SB761
.U62
No. 90-8

LOAN COPY

United States
Department of
Agriculture

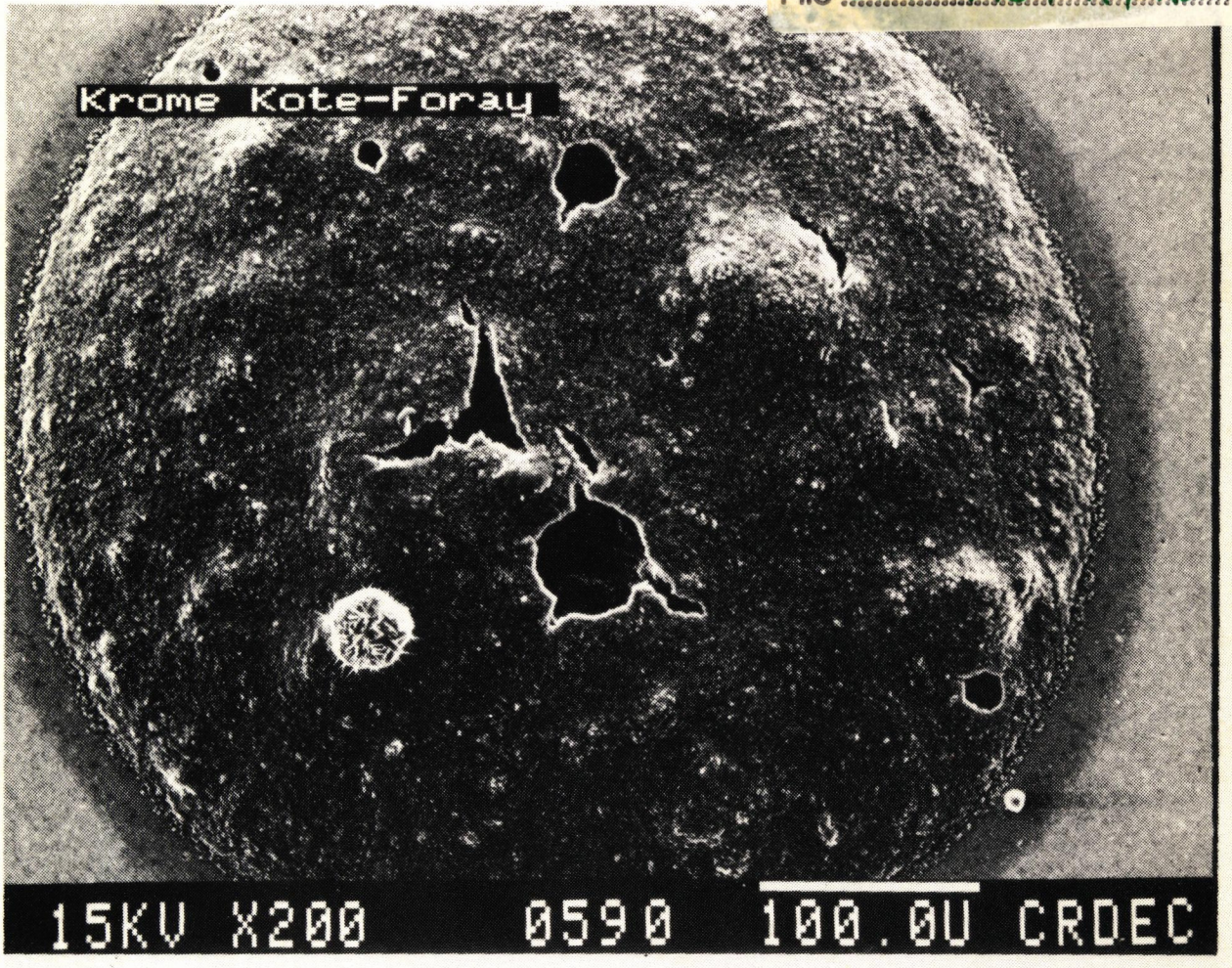
Forest Service

Forest Pest
Management

Davis, CA

SPECTROSCOPICALLY DERIVED SPREADFACTORS FOR DIFFERENT *Bacillus thuringiensis* INSECTICIDAL FORMULATIONS ON PAPER IMPACTION CARDS

FS INFO-Intermountain
MONTHLY ALERT
Edition DEC 1992
Item No. 240
File ~~SDH 7.39 R4 no.90-8~~



EPM 90-8
October 1990

Pesticides used improperly can be injurious to human beings, animals, and plants. Follow the directions and heed all precautions on labels. Store pesticides in original containers under lock and key—out of the reach of children and animals—and away from food and feed.

Apply pesticides so that they do not endanger humans, livestock, crops, beneficial insects, fish, and wildlife. Do not apply pesticides where there is danger of drift when honey bees or other pollinating insects are visiting plants, or in ways that may contaminate water or leave illegal residues.

Avoid prolonged inhalation of pesticide sprays or dusts; wear protective clothing and equipment, if specified on the label.

If your hands become contaminated with a pesticide, do not eat or drink until you have washed. In case a pesticide is swallowed or gets in the eyes, follow the first aid treatment given on the label, and get prompt medical attention. If a pesticide is spilled on your skin or clothing, remove clothing immediately and wash skin thoroughly.

NOTE: Some States have restrictions on the use of certain pesticides. Check your State and local regulations. Also, because registrations of pesticides are under constant review by the U.S Environmental Protection Agency, consult your local forest pathologist, county agriculture agent, or State extension specialist to be sure the intended use is still registered.

Photograph on front cover is a stain on Kromekote paper from a single drop of the biological pesticide FORAY 48B.

AD-33 Bookplate
(1—63)

NATIONAL
AGRICULTURAL
LIBRARY

USDA AGENCY BOOK
Intermountain Station

FPM 90-8
October 1990

SPECTROSCOPICALLY DERIVED SPREADFACTORS FOR DIFFERENT *Bacillus thuringiensis* INSECTICIDAL FORMULATIONS ON PAPER IMPACTION CARDS

Prepared by:

Douglas R. Sommerville
Joseph E. Mata

Resource Directorate
U.S. Army
Chemical Research, Development
and Engineering Center

Aberdeen Proving Ground, MD 21010

Prepared for:

John W. Barry
Project Officer
USDA Forest Service

USDA Forest Service
Forest Pest Management
2121C Second Street
Davis, CA 95616
(916) 758-4600

AD-33 Bookplate
(1—63)

NATIONAL

AGRICULTURAL

LIBRARY

USDA AGENCY BOOK
Intermountain Station

CONTENTS

SECTION	PAGE
1. OBJECTIVE	3
2. BACKGROUND	3
2.1 General Information on the Spreadfactor Relationship	3
2.2 Spectroscopic Approach to Spreadfactor Determination	5
2.2.1 General Description of Methodology and Its Capabilities	5
2.2.2 Evaluation Criteria of Materials for General Spreadfactor Use	6
2.2.3 Evaluation Criteria of Materials for Use with Methodology	6
2.3 Spreadfactor Usage by the USDA Forest Service	7
3. EXPERIMENTAL	7
3.1 Test Solution	7
3.2 Test Procedure	9
3.2.1 Stain Generation for Spreadfactor Determination	9
3.2.2 Solar Degradation Testing of Tracer Dye	10
3.2.3 Stain Sizing	10
3.3 Analytical Procedure	11
3.4 Experimental Design	12
3.4.1 General	12
3.4.2 Solar Degradation Trial	13
4. RESULTS	14
4.1 Spreadfactor Equation for Test Solutions	15
4.2 Solvent Extraction Efficiency	16
4.3 Dye Degradation/Aging	18
4.4 Effect of Droplet Impact Velocity on the Spreadfactor	19
5. CONCLUSIONS	20

PAGE

6. RECOMMENDATIONS 21

ACKNOWLEDGEMENTS 22

LITERATURE CITED 23

APPENDIX A: Instrument Description/Operating Parameters/Calibration 24

APPENDIX B: Raw Experimental Data 26

APPENDIX C: Density Measurements of Thuricide 32LV and Thuricide 32LV/Water 1:1 (mass basis) Mixture 38

APPENDIX D: Sample Calculation to Determine Droplet Freefall Evaporational Losses 40

APPENDIX E: Scanning Electron Microscope Photographs of Test Materials 51-59

SPECTROSCOPICALLY DERIVED SPREADFACTORS FOR DIFFERENT BACILLUS THURINGIENSIS INSECTICIDAL FORMULATIONS ON PAPER IMPACTION CARDS

1. *OBJECTIVE*

Paper witness cards are commonly used to characterize the atmospheric release of aerosol spray droplets. The impacted drops are allowed to dry, and the resultant size distribution of the spot signatures (which are commonly enhanced through the use of tracer dyes) is measured. Through the use of an established spreadfactor relationship, the measured stain size is then converted to the original droplet size. This relationship is determined from controlled laboratory tests and/or actual trial data.

Recently, a novel method for spreadfactor determination has been developed and successfully used with organic liquids by the authors.[1] The method involves the removal of the tracer dye from individual stains through solvent extraction and the subsequent spectroanalysis (both fluorescence and UV/visible) of the resulting solution. The accuracy of the method is dependent on what combination of test liquid/tracer dye/witness card material is being used, which must be determined on a case by case basis.[1]

The objective of the present work is to evaluate the method's accuracy when used with test liquid/tracer dye/witness card materials currently being used by the U.S. Department of Agriculture (USDA) Forest Service in their insecticidal spraying programs. In addition, the possible use of another witness card material with the USDA Forest Service's test liquids/tracer dyes was investigated.

2. *BACKGROUND*

2.1 *General Information on the Spreadfactor Relationship*

The spread factor relationship is known to be dependent upon the physical properties of the fluid, drying conditions, (e.g. temperature/humidity and wind speed) and the type of paper employed. The impact velocity of the droplet on the witness card can also influence the spread factor relationship in some instances, depending on the type of paper used and the physical properties of the fluid.[1,2]

DROPLET FREE FALL/WITNESS CARDS

DEFINITION OF TERMS

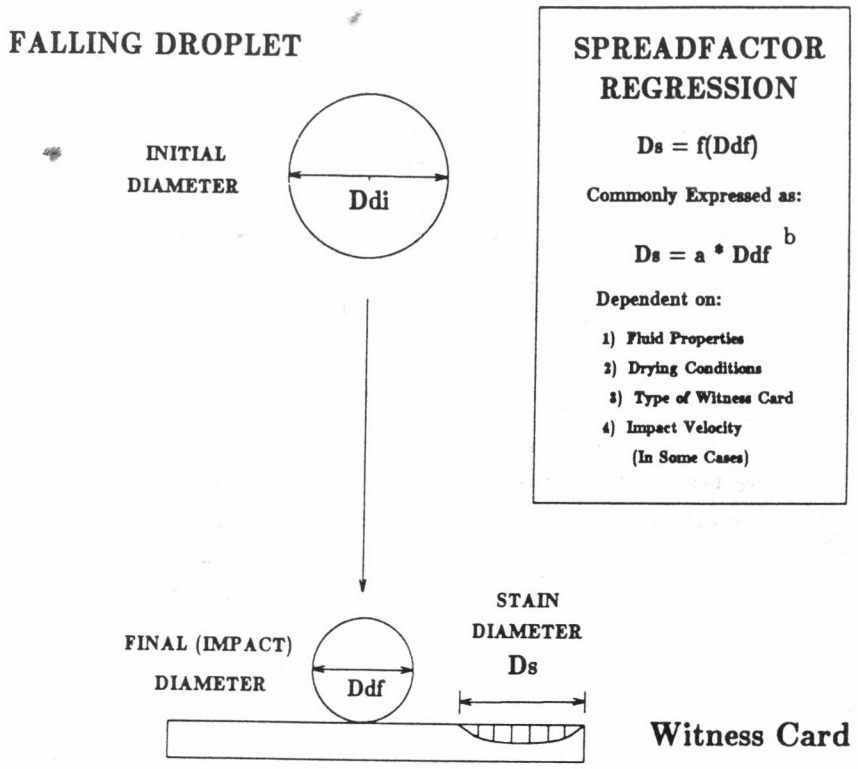


Figure 1 - Definition of terms, droplet free/witness cards.

Bibliographic Records from Title: Spectroscopically derived spreadfactors for different Bacillus thuringiensis
All Records

LDR			02638aam a2200661am 4500
001			vtls000091174
003			USFS
005			20040929151300.0
008			s1990
035			\a (Sirsi) 92 5704
035			\a 92 5704
039		9	\a 200409291513 \b fdernbach \y 200405291511 \z load
040			\a AGO \c AGO
041			\a fen
059			\l PSW \a SB942 \b .S6 1990
059			\l INT \a SD11.A59 \b R4 no. 90-8
100	1	0	\a Sommerville, D. R.
245	1	0	\a Spectroscopically derived spreadfactors for different Bacillus thuringiensis insecticidal formulations on paper impaction cards / \c prepared by Douglas R. Sommerville, Joseph E. Mata ; prepared for John W. Barry.
260	0		\a Davis, CA : \b USDA Forest Service, Forest Pest Management, \c 1990.
300			\a 60 p. : \b ill. ; \c 28 cm.
490	1		\a FPM ; \v 90-8
504			\a Includes bibliographical references (p. 23).
596			\a 4 7
650		4	\a Spectroscopy.
650		4	\a Bacillus thuringiensis.
650		4	\a Biological control agents.
650		4	\a Insecticides.
650		4	\a Spread.
650		4	\a Extension factors.
650		4	\a Laboratory tests.
650		4	\a Spraying.
650		4	\a Accuracy.
700	1	0	\a Mata, J. E.
700	1	0	\a Barry, J. W.
710	1	0	\a United States. \b Forest Service. \b Forest Pest Management.
830		0	\a Forest pest management (Davis, Calif.) ; \v 90-8.
852			~~\a RMRS_OGDEN \c REVIEW \h 11.A59 R4 NO. 90-8 \t 1 \z BOOK~~
852			\a RMRS_OGDEN \c STACKS \h SD11.A59 R4 NO. 90-8 \t 1 \z BOOK
852			~~\a STO \c STACKS \h SB942 .S6 1990 \t 1 \z BOOK~~ Tossed
913			\a NOV 30,1992:12:15
914			\a FSINFO
915			\a APR 3,1995: 0:30
916			\a 105134
917			\a 105133
941			\a WO
942			\a 082. Insects \b 012. Methodology and advanced technology
943			\j INT \e INT9212 \a SO9212
945			\a 921127
949			\a SB942 .S6 1990 \h LOCALMONO \m PSW
949			\a SD11.A59 R4 no. 90-8 \h LOCALMONO \m INT
949			\a 11.A59 R4 no. 90-8 \h LOCALMONO \m DEFAULT
989			\a FSP
989			\a FS
998			\a 11.A59 R4 NO. 90-8 \w LC \c 1 \i 91916-3001 \d 5/29/1999 \l REVIEW \m RMRS_OGDEN \r Y \s Y \t BOOK \u 5/29/1999

The spread factor relationship has been expressed mathematically in many different forms: linear, polynomial, and power law. The first two forms have been used for some applications.[1] However, a power law curve seems to be the more universally accepted expression. A literature search by Cheng (1977) found that the power law was previously used successfully to relate the experimental data for a variety of conditions (eg. droplets impacting coated solid surfaces [lampback, magnesium oxide, or liquid sensitive films]; and papers [Ozalid and filter].[3] The power law even works well for non-newtonian (viscoelastic) droplets/filter paper combinations.[1,4] Hence, the following expression was used to correlate the data in the present work:

$$(1) \qquad D_{df} = aD_s^b$$

where D_{df} is the droplet diameter just prior to impact, D_s is the resulting stain diameter, "a" and "b" are constants dependent on the specific test fluid, ambient experimental conditions, and type of sample paper used (see Figure 1 for definition of terms).

There are two primary sources of error involved in the use of Eq. (1): the experimental determination of the constants a and b; and the actual measurement of D_s. With the latter, the accuracy is greatly influenced by the tracer dye/witness card/test fluid combination used. A poor choice of tracer dye/witness card for a test fluid could result in a poorly defined stain boundary, making it difficult to accurately measure D_s.

The basic approach to spreadfactor determination (solving for the constants a and b) involves the generation of droplets of known size and observing the resulting stain size after droplet impact and drying. D_{df} values have been fixed/calculated in many ways as seen in the following examples:

(a) Vibrating reed apparatus droplet generator--the device calibrated for D_d through photography or use of magnesium oxide (MgO) coated slides.[2]

(b) Direct measurement (with analytical balance) of the droplet's mass immediately after impact with surface (either static or dynamic deposition of droplet on test surface). Known liquid density is then used to calculate D_{df}.[1]

For all of these examples, the calibration stains are normally generated under laboratory conditions. However, the validity of applying laboratory-derived spreadfactors to stains generated in an outdoor field trial may be questionable. Many variables influence the spreadfactor, and it is difficult to exactly match the drying conditions in a laboratory setting with that experienced in a field trial.

2.2 *The Spectroscopically Derived Spreadfactor*

2.2.1 *General Description of Methodology and Its Capabilities*

To solve this problem, a method was developed by the authors to determine the spreadfactor from actual trial stains. The method involved the removal of the tracer dye from individual stains through solvent extraction and the subsequent spectroanalysis of the resulting solution. The dye recovered (assuming 100% extraction efficiency--an assumption that should be verified) allows for the direct determination of D_{di}, the initial droplet diameter (see Diagram 1), since the concentration of the dye is fixed in the test solution. Should evaporation be negligible during free fall, then $D_{di} = D_{df}$, and the spreadfactor equation (Eq. (1)) can be calculated.

It is also possible with this methodology to measure the amount of evaporation experienced by free-falling droplet. To measure evaporation, only D_{di} and D_{df} need to be known (see Diagram 1 for definition of terms):

$$(2) \qquad R_c = 100 \left(\frac{D_{df}}{D_{di}} \right)^3$$

with R_c being the % mass recovered (or 100% minus the mass lossed through evaporation). Both D_{di} and D_{df} can be measured using the methodology: D_{di} from the amount of tracer dye recovered and measured spectroscopically, and D_{df} from the resulting stain size upon droplet impact. An example of the calculations involved is presented in Appendix D.

If droplet evaporation does occur, the actual D_{df} cannot be readily determined from D_{di} measured spectroscopically. A set of calibration stains must be generated at the test site under conditions of no evaporation during free fall (short free fall distance) and allowed to dry under the same conditions as the trial stains. For the

calibration stains, D_{di} will equal D_{df}, which permits the determination of the constants for Eq. (1). Eq. (1) can then be used to find the D_{df} values for the individual trial stains.

2.2.2 *Evaluation Criteria of Materials for General Spreadfactor Use*

The following is a review of the basic evaluation criteria for materials to be used for general spreadfactor work, with emphasis on accurately measuring D_s:

(1) The tracer dye chosen must provide a suitable contrast to the background of the witness card.

(2) The contrast between dye and witness card should be constant over time, since a changing contrast (due to the fading of the dye) could cause variation in D_s values when an image analyzer is used to measure D_s.

(3) The spreading of the test fluid must cease within a reasonable amount of time (about a day). Some low volatility fluid will continue to spread over long periods thus making it difficult to tell when the true D_s has been achieved.

(4) The effects of the droplet's impact velocity on D_s must be taken into account.

2.2.3 *Evaluation Criteria of Materials for Use with Methodology*

The basic premise of the spectroscopic spreadfactor methodology is that an accurate and direct measurement of D_{di} (and in some instances, D_{df} also) is possible. The following is the basic evaluation criteria for accurately measuring D_{di} with the methodology:

(1) It must be possible to totally remove (or at least 95-100% of) the tracer dye present in the witness card using solvent extraction.

(2) The tracer dye must have an appreciable fluorescent or absorbance peak (fluorescence is preferable due to the greater sensitivity) in the solvent of choice.

(3) The stains should have a reasonably long "shelf life", allowing sufficient time to transport the stains from the test site to the laboratory and complete the analytical analysis. The ability to remove stains using solvent extraction is affected by the aging of the dye or dye degradation through solar exposure.

2.3 *Spreadfactor Usage by the USDA Forest Service*

The USDA Forest Service has used witness cards/tracer dyes extensively to monitor the effectiveness of their pesticide spraying operations. In the past, they have used the previously explained methods of spreadfactor determination (see Section 2.1). Due to the known USDA Forest Service interest in spreadfactor methods, the opportunity was presented to the authors to test the effectiveness (and thus the versatility) of the spectroscopic spreadfactor methodology using USDA Forest Service materials. These materials were provided by the USDA Forest Service for use by the authors.

3. *EXPERIMENTAL*

3.1 *Test Solution and Witness Card Materials*

Two different brands of insecticidal formulations were used in the present work: Foray^{TM} 48B Flowable Concentrate, manufactured by Novo Laboratories, Inc., Danbury, CT; and Thuricide® 32LV Microbial Insecticide Concentrate, manufactured by Sandoz, Inc., San Diego, CA. The active ingredient for both products is *Bacillus thuringiensis* Berliner variety *kurstaki* (or BTK), comprising 15% of the material in Foray 48B and 1.6% of Thuricide 32LV. The remaining material for both solutions is described by the manufacturers as inert ingredients. Samples of both products were supplied by the USDA Forest Service. The products can be applied in both their pure form or water diluted mixture. For the present study, solutions of pure Foray and Thuricide were used, as well as an 1:1 mixture (mass basis) of Thuricide and distilled water.

Rhodamine WT Liquid (also supplied by the USDA Forest Service) was added to each solution. Rhodamine WT Liquid is a red, water soluble, fluorescent dye (CAS No. 703-010-27), manufactured by Keystone Aniline Corp., La Mirada, CA. Rhodamine WT has an optimum excitation wavelength of 556 nm and an optimum emission wavelength of 580 nm. The nominal dye concentration for all of the test solutions was 1 wt%. The USDA Forest Service normally use a 0.1 wt%

concentration for their purposes,[5] but initial work in the present study showed that 0.1 wt% would greatly reduce the sensitivity of the spectroscopic spreadfactor methodology.

The exact dye concentration in each solution was determined through fluorescent measurements. The fluorescence intensity of the dye (at the above wavelengths) is approximately 0.14 intensity units per ng of dye/ml solution (based upon the fluorescence calibration curves made).

Two types of witness cards were used in the present study: Kromekote Cov. White C2S (supplied and extensively used by the USDA Forest Service),[6] and Bactkote #1120 Membrane Filter Paper (pore size of 0.45 μm), manufactured by Schleicher and Schuell of Keene, NH.

Kromekote cards are white cards with a glossy surface (both sides). The test solutions used are not readily absorbed by the paper itself, but rather, the solution upon drying forms a dry crust on the paper surface. Over a period of time, it is possible that some of the tracer dye could be lost through the "flaking off" of the stain over time.

Bactkote cards are composed of a dual layer of uniformly thin and porous cellulose nitrate film (membrane) on top of a heavy filter paper (both being fused together). The nitrate film has a sponge like structure with a well defined pore size. Bactkote paper was originally designed to filter out small particulates from liquid solutions.

In the present work, the membrane side of the Bactkote paper was used to capture the falling droplets. The test solution is readily absorbed by the membrane layer of the card, but the membrane does not readily promote liquid/droplet spreading in comparison with other paper types (ie. Kromekote, Whatman filter paper, etc.). Bactkote paper has been successfully used by the authors in sampling organic liquid droplets (unpublished work).

Photographs were taken of some of the materials (Foray/Rhodamine WT stains on both Bactkote and Kromekote papers, as well as the papers separately) using a Scanning Electron Microscope (SEM). Features such as individual BTK spores and the surface structure of the papers can be seen. The photographs with

accompanying descriptions, as well as the procedures and equipment employed, are presented in Appendix E.

3.2 *Test Procedure*

3.2.1 *Stain Generation for Spreadfactor Determination*

Three different methods were used to generate droplets from each test solution:

(1) Wire Drop Technique (WDT)--Static deposition of sessile liquid droplet suspended from the end of thin wire to the surface of the witness card. Each drop so formed was weighted by difference using an analytical mass balance with the witness card being on the scale when the droplet was deposited. Due to the difficulty in accurately measuring droplet mass values less than 1.0 mg, the lower limit of attainable D_d values is approximately from 800 to 1000 μm. This technique was used to generate droplets for:

(a) Use in the studies of the Rhodamine WT Liquid's solar stability.

(b) To study droplet velocity (ie. zero velocity) effects on D_s.

(c) Comparison purposes--check on solvent extraction efficiency.

(2) Common Household Pump Spray (PS)--The hand-held, finger operated device was chosen due to ease of operation. The pump spray was used for the majority of stains created. The pump was position approximately 10 feet above the witness cards. A wide range of D_d values, from 200 to 7000 μm were obtained with the pump spray. In the manner used in the present study, the generated droplets were assumed to be traveling at their terminal velocity.

(3) Compressed Air Spray with Nozzle (CAS)-- A Spraying Systems Air Atomization Nozzle Set-up #E15B was mounted on the end of a compressed air line. Approximately 8 psi of pressure was used to aerosolize the test solution as it left the nozzle. The CAS was used originally to generate a greater number of smaller diameter droplets (D_d values in the range of 200 to 700 μm) than was

possible with the PS. The nozzle was positioned 10 feet above the witness card for trial SS9345TH (Thuricide) and 2 feet for trial SS9345F (see Section 3.4.1 and Table 1). Clogging of the nozzle and droplet drift forced the use of a shorter height for SS9345F (Foray 48B). It was originally assumed that from both spray heights the droplets were at their terminal velocity upon impact. However, subsequent test results suggest that this was not truly the case with SS9345F (see Section 4.4 for a more complete description).

For both PS and CAS, the sprayer was positioned directly above the witness cards. The witness cards where placed on top of a piece of sheet metal and secured with tape prior to being sprayed with the test solution. Upon the completion of the spraying, the impacted cards were taken to a dark location and allowed to dry overnight indoors. The room temperature was approximately 20^{o}C.

3.2.2 *Solar Degradation Testing of Tracer Dye*

To test the solar stability of Rhodamine WT Liquid, a series of stains were created using the WDT with the pure Thuricide test solution onto Kromekote paper. The droplets were carefully weighted by difference on an analytical balance.

Half of the stains were then exposed to four hours of simulated solar radiation using an Oriel Corp. Model 81100 Solar Simulator using an Air Mass 2 Direct Filter. This filter simulates the solar spectrum expected on the earth's surface when the sun is 60^{o} above the horizon. The intensity of the simulated radiation is approximately 1.5 times that expected under natural conditions (ie. 4 hours of simulated radiation is equivalent to 6 hours of actual solar radiation). The other half of the stains were set aside in a dark location to act as blanks.

After being exposed under the simulator, the exposed stains were moved to a dark location. At interval of 1 week, 2 weeks, and 4 weeks, individual stains from the exposed and unexposed batchs were randomly selected and then analyzed to see if the fluorescence intensity of the Rhodamine WT Liquid had been diminished due to the simulated solar exposure (see Section 3.3 for the Analytical Procedure used).

3.2.3 *Stain Sizing*

Upon drying, the stains were sized on a Quantimet 920 Image Analyzer, manufactured by Cambridge Instruments, Ltd, Cambridge, United Kingdom. With the lens/camera configuration used, the error in D_s was estimated to be $\pm$ 35 μm.

3.3 *Analytical Procedure*

To determine the amount of dye present in the individual droplet stains, stains were cut out of the witness cards and individually placed in a 10 ml volumetric flask. The flask was then filled to the mark with distilled water (having a fluorescence background of less than 1 ppb as quinine sulfate) and placed in a ultrasonic bath for 15 minutes to promote the extraction of the dye from the card.

For stains smaller than 600 μm in diameter, several stains of approximately the same diameter were placed in one flask in order to obtain a higher fluorescence intensity. The stains were selected by comparing their D_s values. The selection was made such that the variation in the D_s values was $\pm$ 10 to 20 μm. For subsequent calculations, the D_s value for the sample was obtained by taking the average of all the stains' D_s values present in the sample. An average D_{df} value was determined by taking the total test solution mass as measured by fluorescence (see below) and then dividing by the total number of stains used in the sample.

Stains created with the WDT were used to determine the efficiency in recovering the dye from the card. The appearance of either dye degradation and/or the permanent setting of the dye in the card can effect the efficiency of dye recovery. These two phenomena are primarily dependent on the stain's age, previous solar exposure, and the type of witness card used. Under ideal conditions (ie. solvent extraction performed 24 hours after stain has dried with no solar exposure), the present study found that at least 99 wt% of the dye was successfully extracted from the Kromekote paper. Several tests were conducted to specficially evaluate the solvent extraction efficiency and are discussed in Section 4.2.

Upon removal from the bath, the aqueous solutions were then spectroanalyzed using 3 ml quantities. Solutions that gave off too much fluorescence intensity were further diluted to obtain a fluorescence intensity within the calibration range of the spectrophotometer.

A Perkin Elmer Model LS-3B Fluorescence Spectrophotometer was used to measure the fluorescence of the Rhodamine WT Liquid. Prior to analyzing the dye stains, calibration curves were made using water/dye solutions using prepared solutions of known composition (see Appendix A for the exact procedure employed). The wavelengths used to measure fluoresence, as well as other pertinent instrument parameters, are listed in Appendix A. Possible background fluorescence interference due to residue from the witness card was also tested for, and it was found to be negligible for the range of Rhodamine WT concentrations employed in the present work.

3.4 *Experimental Design*

3.4.1 *General*

A total of 11 trials were conducted, involving a total of 256 samples (746 individual stains). One stain per sample was the norm, but for the smaller diameter stains (see Section 3.3), up to 18 individual stains were used per sample. D_s values ranged approximately from 125 to 5400 μm, and the corresponding D_d values ranged approximately from 125 to 2900 μm.

Several variables of interest to the authors and/or the USDA Forest Service were investigated in these trials (with some trials involving more than one variable):

(1) Straight-forward determination of the spreadfactor relationship for all three test solutions (at least one trial per solution): using USDA Forest Service materials; with droplets traveling at their terminal velocity; and under conditions of no freefall evaporation (4 trials total).

(2) Comparison of the relative merits of Kromekote and Bactkote papers as witness cards for the USDA Forest Service formulations (6 trials total).

(3) Evaluation of the Rhodamine WT Liquid's resistance to degradation from aging and exposure to solar radiation (1 trial).

(4) Evaluation of the efficiency of the removal of the dye from stains through solvent extraction (6 trials total).

(5) Evaluation of the effects of the droplet's impact velocity upon the spreadfactor relationship (data from all 11 trials).

Table 1 gives a summary of all the trials conducted. The type of droplet generation, test solution, witness card material, and the number of stains/samples used are given:

TABLE 1

Summary of Trials Conducted
Number of Samples/Stains Per Variable

		Test Solution			Witness Card	
Test Designation	Droplet Generation	Thuricide	Thuricide/ Water Mix	Foray	Bactkote	Kromekote
SS9331TH	PS	30/49	0	0	10	20
SS9331F	PS	0	0	30/44	10	20
SS9335TH	WDT	15	0	0	7	8
SS9335F	WDT	0	0	15	7	8
SS9339TH	WDT	20	0	0	10	10
SS9339F	WDT	0	0	16	8	8
SDT9333TH	WDT	42	0	0	0	42
SS9345TH	CAS	15/122	0	0	0	15
SS9345F	CAS	0	0	15/158	0	15
SS0002F	PS	0	0	24/119	0	24
SS0036THW	PS	0	34/146	0	0	34

Key:
1.) Single number--Total number of samples using attribute
2.) 1st number/2nd number--number of total samples with attribute/number of total stains used with attribute
3.) Test Designation--first set of letters in code
SS--Stain Series
SDT--Solar Degradation Test
4.) See Section 3.2.1 for Abbreviations for Droplet Generation

3.4.2 *Solar Degradation Trials*

SDT9333TH (see Table 1) was conducted to test the effects of solar exposure and aging on the ability to recover Rhodamine WT Liquid from Kromekote paper.

Kromekote data from SS9335TH was also used in conjuction with the data from SDT9333TH in the final data analysis.

A total of 50 stains, divided into 7 sets of data (including SS9335TH, Set B), were made. 3 sets were exposed to solar radiation for 4 hours (see Section 3.2.2 for procedure used), while the other four were kept in a dark location after being made. The storage times represent are 4, 7, 14, and 28 days, with each storage time being represented by an exposed and unexposed set of data , except for the storage time of 4 days (unexposed–SS9335TH, Kromekote paper data).

It should be noted that there are many possible causes for the apparent loss or degradation of dye and/or dye intensity: chemical breakdown of dye due to weathering, age, or solar exposure; flaking off of the dye from the witness card; the setting of the dye into the witness card over time (causing inefficient solvent extraction); etc. For the purposes of the present work, the term "solar degradation" shall be used for dye degradation as a direct result of the dye's exposure to solar radiation. The term "dye aging" shall refer to degradation resulting from all other causes, though primarily those causes whose effects are not readily apparent expect over a period of time.

4. *RESULTS*

The results of the present study are divided up into four categories:

(1) Spreadfactor Equations for Test Solutions

(2) Solvent Extraction Efficiency

(3) Dye Degradation/Aging

(4) Effects of Droplet Impact Velocity on the Spreadfactor

The following sections present these results in both enclosed figures and tables, with accompanying statistical analyzes. The raw data is presented in Appendix B

In addition, in order to convert the spectroscopically measured droplet mass into the corresponding diameter value, the density of the solution has to be known. The manufacturer's product literature on Foray 48B gave a density value of 1.16 g/ml for Foray 48B (no temperature given in literature for this value--room temperature assumed).[7] No density values were available for Thuricide 32LV. Measurements were taken by the authors (see Appendix C for details) and density values of 1.15 and 1.07 g/ml were found for pure Thuricide 32LV and 1:1 mix (mass basis) of Thuricide/Water. These three density values were used in all subsequent calculations.

4.1 *Spreadfactor Equations for Test Solutions*

Figures 1-3 present the results from Tests SS9331TH, SS9345TH, SS0036THW, SS9331F, and SS0002F for the spreadfactor of Thuricide 32LV, Thuricide/Water 1:1 mixture (mass basis), and Foray 48B on Kromekote paper. Both individual data points and the least square fit of the data (including 95% confidence levels) are shown in the figures. It is highly recommended that the curves not be extrapolated beyond the limits set by the experimental data. This is particularly true at the low end of D_d and D_s values due to the increasing influence from possible freefall droplet evaporation (which is difficult to prevent under the conditions of the present study for D_d values less than 200 μm).

A least square fit of the data was performed (using a mathematical/statistical computer package),[8] with $\log_{10} D_{di}$ (D_{di} equals D_{df} since there is no evaporation) as a linear function of $\log_{10} D_s$ (with a slope of b and y-intercept of $\log_{10} a$). The resulting values for the constants a and b for the three test solutions are presented in Table 2.

Based upon the results of the least square fit, error estimates for D_d were also made for the constants of Eq. (1) and measured D_s values. These estimates are also shown in Table 2. In addition, the correlation coefficient squared values for all three lines are greater than 99.4%.

The effects that dilution of Thuricide 32LV has on the spreadfactor can be determined through the comparison of Figures 1 and 2. The two spreadfactor curves for Thuricide (Figure 1) and Thuricide/water (Figure 2) are statistically different from each other (ie. points from one curve do not fall in the 95% confidence range of the other curve). Both curves do have the same slopes, but the

Thuricide/water mixture curve is slightly offset to the right of the Thuricide curve. The greater spreading of the Thuricide mixture for a particular D_d value is probably due to the lower solution viscosity obtained through the dilution.

TABLE 2

Values for Spreadfactor Regression Constants {Eq. (1)}
Power Law Curve of D_d as Function of D_s

Test Solution	*a* {y-intercept}	*b* {slope}	% Error in D_d from Measured D_s
Thuricide	2.6 ± 0.2	0.76 ± 0.01	6.5
Thuricide/ Water Mix	2.3 ± 0.2	0.76 ± 0.01	4.9
Foray	5.3 ±0.2	0.69 ± 0.007	4.3

4.2 *Solvent Extraction Efficiency*

A comparison of the solvent extraction efficiency for removing Rhodamine WT Liquid from the two types of witness cards used in the present study (Bactkote and Kromekote) is shown in Figures 4 (Low Region) and 5 for Foray 48B and Figures 6 (Low Region) and 7 for Thuricide 32LV. All the samples were generated with the WDT (from Tests SS9335TH, SS9335F, SS9339TH, SS9339F), and all stains were analyzed 2-4 days after being created. An analytical balance was used to measure the original mass of each droplet.

The following relation was used in the reduction of the data:

$$(3) \quad R_{mass} = M_{fluor} \; / \; M_{cahn}$$

where, M_{fluor} is the droplet mass as determined from the fluorescence measurement of the dye recovered through solvent extraction; M_{cahn} is the droplet mass as measured with the Cahn Microbalance at the creation of the droplet with the WDT; R_{mass} is the ratio of recovered dye mass (proportional to M_{fluor}) to that of the original dye mass (proportional to M_{cahn}).

For convenience, M_{fluor} and M_{cahn} were used instead of the actual dye mass values involved. Perfect dye recovery is respresented by $R_{mass} = 1.0$.

In each figure is plotted the 100% recovery line (ie. $M_{fluor} = M_{cahn}$), as well as the 80% and 60% recovery lines ($M_{fluor} = 0.8M_{cahn}$ and $0.6M_{cahn}$, respectively) for comparison. The figures show that obtaining 100% dye recovery from the Kromekote paper was consistently achieved, where as the same was the not the case with the Bactkote paper. With the Bactkote paper, recovery values were consistently below 60% (with a couple of exceptions) with the dye in Foray 48B solutions and from 60 to 90% (majority of values) for the dye in Thuricide 32LV solutions.

The poor showing of the Bactkote paper is probably due to a contrast in the paper's designed application and how it was actually used. Bactkote paper was designed for the filtering (and hence retention) of fine particulates. Both Thuricide and Foray are colloidal suspensions, and particulates (possibly containing an appreciable amount of the tracer dye) from these solutions could be readily captured by the Bactkote paper. There is also the possibility that the membrane layer of the Bactkote paper has a strong affinity for Rhodamine WT Liquid as well.

The SEM photographs in Appendix E, Figures E3 (Bactkote surface) and E4 (Kromekote surface), give a clear picture of the paper and pesticide solution qualities (discussed above) which influence the dye extraction efficiency. The membrane layer of the Bactkote paper is very rough and porous (sponge-like). The Kromekote paper is very densely packed with small imperfections (holes) in the paper about 1 μm or smaller in diameter.

Examination of Figures E6 and E7 show that the filler material of the pesticide is granular in nature and approximately 1 μm in size. The BTK spores (see Figure E5) range from 40 to 60 μm in diameter. The size range of the particulates would prevent ready capture by the densely packed Kromekote paper, but not in the case of porous Bactkote paper. Paper qualities hindering particulate particulate capture could simultaneously promote efficient extraction, which may explain the high extraction efficiency obtained with Kromekote paper.

Solvent extraction efficiency for stains (made on Kromekote paper) kept in storage longer than 4 days was investigated as part of the testing for dye

degradation/aging in Section 4.3.

4.3 *Dye Degradation/Aging*

A total of 44 out of 50 stains from SDT9333TH and SS9335TH, set B (see Table 1) were used to evaluate the effects of dye degradation and/or aging. The exact procedure employed for the trials is described in Section 3.2.2, and the experimental design in Section 3.4.2. The stains were made with the Thuricide 32LV/Rhodamine WT Liquid test solution on Kromekote paper. Eq. (3) (see Section 4.2) was used in the reduction of the data.

Two variables were varied in the tests, with at least six stains per set of variables: storage time (4, 7, 14, and 28 days) and exposure to solar radiation (0 and 4 hours). A total of seven variable sets are used. The mean and standard deviation were taken of the individual R_{mass} values per set of variables. The results are shown in Table 3, and the individual results are presented in Figure 8.

TABLE 3

Solar Degradation/Aging Results

Average R_{mass} Values as a Function of Storage Time and Solar Exposure For Rhodamine WT Liquid (in Thuricide 32LV) on Kromekote Paper

	Exposure Time			
Storage Time (Days)	Zero Hours		Four Hours	
4	1.04	± 0.03		
7	1.02	± 0.02	1.02	± 0.03
14	0.97	± 0.03	0.96	± 0.03
28	0.93	± 0.03	0.90	± 0.05

No dye mass loss is represented by $R_{mass} = 1.0$. However, average values of R_{mass} up to 1.04 were obtained. This was probably due to a systematic error in either/both the fluorescence spectrophotometer calibration curve or in the operation of the analytical balance. Thus, all dyes losses should be compared with

$R_{mass} = 1.04$.

A comparison of the R_{mass} values in Table 3 shows that dye aging (see Section 3.4.2 for definition) is the predominant mechanism of dye loss. After 14 days of storage time, about 5-7% of the dye had been "lost," when compared to R_{mass} value of 4 days. After 28 days, the loss grows to 9-12%. This trend is evident in Figure 8. However, solar degradation is not an appreciable factor in dye loss, for there is no significant difference in the R_{mass} values between the exposed and unexposed stain sets.

Attempts have been made to develop a model to fit the data, but there is too much scatter in the data. Only a qualitative evaluation can be made from the data (as mentioned above).

4.4 *Effects of Droplet Impact Velocity on the Spreadfactor*

In Figure 9, a comparison is made between spreadfactors obtained from three different droplet impact velocities, zero (using WDT), terminal (with PS), and greater than terminal (with CAS). All stains were made with Foray 48B on Kromekote paper (Trials SS9331F, SS9335F, SS9339F, SS9345F, and SS0002F). The WDT and CAS data is represented by individual data points, while the PS data is represented by the least square fit curve (including 95% confidence level lines) of the data shown in Figure 3 for Foray 48B droplets. Figure 9 shows that significant deviations in the spreadfactor can be expected with varying changes in the falling droplet's velocity at the point of impact with the witness card.

The droplets having zero velocity (WDT) produced a spreadfactor curve with a greater slope, as well as being above, the curve for the terminal velocity droplets. The differing slopes of the WDT and PS droplets indicate a possible intersection of the curves for the smaller droplets sizes. Difficulty in accurately measuring the mass of WDT droplets for D_d values less than 700 μm prevents ready confirmation of this. However, such an agreement between the WDT and PS curves for D_d less than 700 μm would imply that the droplet impact velocity may not be a factor for the spreadfactor of the smaller diameter droplets.

Originally when the CAS was used to generated droplets (see Section 3.2.1), it was assumed that the droplets were traveling at their terminal velocity upon impact

with the witness cards. However, subsequent comparison of the CAS data with the PS data for Foray 48B shows a significant (being outside the 95% confidence curves of the PS curve) deviation, with the CAS curve being parallel to, but to the right of, the PS curve.

It was concluded that the additional spreading of the CAS droplet in comparison to a PS droplet of the same D_d was probably the result of the greater impact velocity (and hence kinetic energy) of the CAS droplet (since a short fall distance and compressed air nozzle was used--see Section 3.2.1). Temperature effects were ruled out since no temperature variation was noted during the creation and drying of both stain types.

The same phenomena was not noticed for the CAS data with the Thuricide 32LV solution (trial SS9345TH). In fact, the Thuricide CAS data was incorporated into the linear regression analysis to obtained the spreadfactor for the solution. The lack of any statistical difference between the PS and CAS data for Thuricide is probably due to the greater height used to spray the Thuricide CAS droplets (10 feet) in comparison to the that used for the Foray CAS droplets (2 feet) (see Section 3.2.1). The greater height would give the Thuricide CAS droplets a sufficient "stopping" distance--the distance required to slow from greater than terminal velocity (when coming out of the nozzle) to terminal velocity. 2 feet may not be a sufficient "stopping" distance for Foray CAS droplets.

5. *CONCLUSIONS*

The droplet spreadfactor relationship determined through the use of fluorescent dye tracers is accurate and reliable for the USDA Forest Service material tested. The methodology can determine the spreadfactor relationship from stains directly from an actual field dissemination trial without having to attempt to approximate field conditions in the laboratory.

Possible hinderances to the use of the methodology, such as losses of the dye due to aging or solar exposure, were found to be negligible if the stains are promptly analyzed (within 2 weeks) after being made. After 2 weeks of storage, incomplete dye extraction becomes a significant problem (less than 90% recovery).

Velocity effects are significant. The effects are due more to the impact of the droplet rather than deformation of the droplet during freefall. Thus, when making calibration curves for the spreadfactor, care must be taken to produce droplets that will impact with the witness cards at the same velocity as actual trial droplets (terminal velocity).

The use of Bactkote paper, though successfully used with organic liquids,[1] is not a good choice as a witness card for USDA Forest Service materials. As was shown, there were problems in obtaining 100% extraction of the dye from the Bactkote paper, primarily the result of the retention of particulates (in the stains) from the pesticidial solutions by the paper (see Section 4.2 for more complete description of problem). Possibly, Bactkote paper could still be used with USDA Forest Service Materials if the efficient removal of particulates could be accomplished.

The dilution of Thuricide 32LV does slightly affect the spreadfactor relationship, for the range of 100% to 50% (mass basis) Thuricide in an aqueous solution. Due to the similar nature of Foray 48B to that of Thuricide (colloidal suspensions), dilution of Foray 48B may produce the same type of change in the Foray spreadfactor.

6. *RECOMMENDATIONS*

The accuracy of the method is highly dependent on the ability to obtain 100% recovery of the tracer dye from each stain. Thus, it is recommended that all stains be analyzed within 2 weeks of being made in order to avoid large dye losses due to aging effects. Even though there is no evidence that solar exposure will cause dye degradation in Rhodamine WT Liquid, it is recommended that stains be kept out of the sun as much as possible as a precaution. Also, care should be taken in the handling and storage of the cards to avoid the inadvertent "flaking" off of the stains when using Kromekote paper.

When generating calibration stains, it is recommended that a hand sprayer, rather than the WDT, be used to generated the droplets due to the effects of the impact velocity on the spreadfactor. If the WDT must be used, then a substitution from Kromekote paper to a more absorbent paper (eg. Whatman Filter paper) must be made. Highly absorbent paper minimizes the effects of the droplet's impact velocity on the spreadfactor.[1]

Before using the methodology with any witness card/tracer dye/test fluid combination, it is recommended that preliminary trials similar to that conducted in the present work be performed. As seen with the dye extraction efficiency results for the Bactkote paper, compatibility among the above three components should not be assumed.

ACKNOWLEDGEMENTS

The authors wish to acknowledge with sincere appreciation the laboratory assistance of Paulette Jones and Angela R. Farenwald, both of the Chemical Research, Development and Engineering Center (CRDEC), Aberdeen Proving Ground, MD.

The authors also wish to acknowledge Dorothea A. Paterno, CRDEC, for her SEM photographs of the test materials used in the present study.

LITERATURE CITED

1. Sommerville, D. R., and J. E. Matta, *"A Method for Determining Droplet Size Distributions and Evaporational Losses Using Paper Impaction Cards and Dye Tracers."* CRDEC-TR-xxxxxx, U.S. Army, Chemical Research, Development and Engineering Center (CRDEC), Aberdeen Proving Ground (APG), MD, in publication, UNCLASSIFIED REPORT.

2. Barry, John W., et. al., *"Methods For Sampling and Assessing Deposits of Insecticidal Sprays Released Over Forests".* **Technical Bulletin No. 1596**, US Department of Agriculture (USDA) Forest Service, Davis, CA, November 1978.

3. Cheng, L. *"Stain Method for Measurement of Drop Size,"* **Environmental Science and Technology. 11** (2): 192-194, 1977.

4. Matta, J. E., R. P. Tytus, and J. L. Harris, *"Aerodynamic Atomization of Polymeric Solutions,"* **Chemical Engineering Commun. 19:** 191-204, 1983.

5. Barry, John W., **Private Communication.** with Joseph E. Matta (author), USDA Forest Service, Davis, CA, September 1, 1989.

6. Kautz, J. and R. B. Ekblad, **Analysis of Spray Deposit Cards Sensitive to Nondyed Mixes.** Report for Project #TI 3E32P44, Spray Deposit Assessment, USDA Forest Service, Missoula, MT, 1984.

7. *"Foray 48B Flowable Concentrate,"* Product Description Sheet distributed by Novo BioKontrol, a Division of Novo Laboratories, Inc., Danbury, CT, ca. 1989.

8. *"Minitab Release 5.1,"* **Minitab, Inc.**, 1985.

APPENDIX A

INSTRUMENT DESCRIPTION/OPERATING PARAMETERS AND DYE CHARACTERISTICS

A-1. *INSTRUMENT DESCRIPTION/OPERATING PARAMETERS*

Perkin-Elmer Model LS-3B Fluorescence Spectrophotometer

The LS-3B Spectrophotometer is a fluorescence spectrophotometer with separate excitation and emission scanning monochromators. It uses a pulsed xenon flash tube as a source, and a spectrally compensated signal is obtained by directing the reference beam through the a built-in triangular cell containing a solution of Rhodamine 101 dye. The whole system operates in a pulse mode with a short duty cycle; the source is pulsed and the detectors are gated at line frequency.

In the present work, the following instrument parameters were used for measuring dye fluorescence:

a. Slit width--
   Emission: 10 nm
   Excitation: 10 nm
b. Response Time: 4.0 seconds
c. Integration Time: 8.0 seconds
d. Cell Pathlength: 10 mm
e. Sample Temperature: 25°C
f. Sample Volume: 25 ml

A-2. *INSTRUMENT CALIBRATION PROCEDURES*

A-2.1 *Calibration Curve--Dye Concentration Versus Instrument Reading*

In order to determine the amount of dye recovered from a stain (and hence determine the mass of the original droplet), a calibration curve first had to be prepared for use with the Perkin-Elmer spectrophotometer.

A-2.2 *Quality Assurance Measures Taken for the Analytical Instruments*

The fluorescent intensity that is emitted by (and detected for) a particular sample in a fluorescence spectrophotometer is proportional to the energy output from the instrument's light radiation source. If this is not accounted for by the use of standards or very frequent up-grading of calibration curves, errors in measuring fluorescence could easily occur.

In order to analysis two or more fluorescence data sets taken on different days, the effects of the changing energy output of the source had to be accounted for. This was done through the use of solid fluorescence standards provided by the Perkin-Elmer Company for use in their instruments. These standards consisted of polymethylmethacrylate (PMMA) blocks impregnated with various fluorescent dyes. The PMMA provides stability to the dyes, so that any changes in fluorescence intensity is instrumental rather than chemical in nature.

Thus, the PMMA blocks were used to "correct" sample measurements to that of a calibration curve by using the ratio of the intensity reading of the PMMA blocks taken at the time of the sample to that taken at the time of the calibration curve. By taken reading of the PMMA blocks frequently (between each or every other sample), even source variations over a short time period could be effectively account for in their effect on intensity readings.

APPENDIX B

Experimental Data
(see also Table 1)

TABLE B1.1

Test: Stain Series 9331TH, Set A
Storage Time: 3 days
Test Fluid: Thuricide 32LU
Witness Card Material: Bac-T-Kote Membrane #1121
*Generation Method: PS

No. stains used in sample	Ds (um)	Ddf (um)
5	277	212
5	370	259
3	459	327
1	685	436
1	794	474
1	1078	590
1	1268	652
1	2525	988
1	3226	1165
1	5599	1666

TABLE B1.2

Test: Stain Series 9331TH, Set B
Storage Time: 3 days
Test Fluid: Thuricide 32LU
Witness Card Material: Kromekote Cov. White C2S
*Generation Method: PS

No. stains used in sample	Ds (um)	Ddf (um)
4	327	242
3	531	305
2	747	428
1	939	517
1	1166	578
1	1469	653
1	2077	896
1	3167	1234
1	4461	1583
1	6947	2163

TABLE B1.3

Test:	Stain Series 9331TH, Set C
Storage Time:	9 days
Test Fluid:	Thuricide 32LU
Witness Card Material:	Kromekote Cov. White C2S
*Generation Method:	PS

No. stains used in sample	Ds (um)	Ddf (um)
3	603	366
2	520	324
1	815	443
1	1072	542
1	1169	600
1	2386	1054
1	2794	1156
1	3687	1398
1	5293	1818
1	7515	2382

TABLE B2.1

Test:	Stain Series 9331F, Set A
Storage Time:	3 days
Test Fluid:	Foray 48B
Witness Card Material:	Bac-T-Kote Membrane #1121
*Generation Method:	PS

No. stains used in sample	Ds (um)	Ddf (um)
1	7632	1774
1	5415	1878
1	3842	1431
1	3298	1355
1	2399	1062
1	1503	777
1	1275	718
3	797	501
6	467	347
5	354	278

TABLE B2.2

Test:	Stain Series 9331F, Set B
Storage Time:	3 days
Test Fluid:	Foray 48B
Witness Card Material:	Kromekote Cov. White C2S
*Generation Method:	PS

No. stains used in sample	Ds (um)	Ddf (um)
2	395	316
2	518	375
2	621	437
1	859	555
1	1250	707
1	1632	866
1	2628	1057
1	3300	1364
1	5213	1868
1	6553	2185

TABLE B2.3

Test:	Stain Series 9331F, Set C
Storage Time:	9 days
Test Fluid:	Foray 48B
Witness Card Material:	Kromekote Cov. White C2S
*Generation Method:	PS

No. stains used in sample	Ds (um)	Ddf (um)
2	327	274
1	799	548
1	725	520
1	1201	704
1	1470	796
1	1321	748
1	2864	1265
1	3648	1468
1	4038	1529
1	4955	1890

TABLE B3.1

Test:	Stain Series 9335TH, Set A
Storage Time:	4 days
Test Fluid:	Thuricide 32LU
Witness Card Material:	Bac-T-Kote Membrane #1121
*Generation Method:	WDT

No. stains used in sample	Ds (um)	Ddf (um)
1	848	802
1	1115	932
1	897	779
1	794	789
1	788	839
1	797	767
1	1179	895

TABLE B3.2

Test:	Stain Series 9335TH, Set B
Storage Time:	4 days
Test Fluid:	Thuricide 32LU
Witness Card Material:	Kromekote Cov. White C2S
*Generation Method:	WDT

No. stains used in sample	Ds (um)	Ddf (um)
1	1573	895
1	1196	670
1	1644	916
1	1412	806
1	1425	804
1	1433	805
1	1226	702
1	1761	957

TABLE B4.1

Test:	Stain Series 9335F, Set A
Storage Time:	4 days
Test Fluid:	Foray 48B
Witness Card Material:	Bac-T-Kote Membrane #1121
*Generation Method:	WDT

No. stains used in sample	Ds (um)	Ddf (um)
1	809	730
1	932	646
1	779	785
1	880	636
1	822	592
1	695	543
1	930	752

TABLE B4.2

Test:	Stain Series 9335F, Set B
Storage Time:	4 days
Test Fluid:	Foray 48B
Witness Card Material:	Kromekote Cov. White C2S
*Generation Method:	WDT

No. stains used in sample	Ds (um)	Ddf (um)
1	1439	978
1	1207	826
1	1369	926
1	1501	1048
1	1344	877
1	1298	898
1	1221	848
1	1407	915

TABLE B5.1

Test:	Stain Series 9339TH, Set A
Storage Time:	2 days
Test Fluid:	Thuricide 32LU
Witness Card Material:	Kromekote Cov. White C2S
*Generation Method:	WDT

No. stains used in sample	Ds (um)	Ddf (um)
1	2849	1480
1	2320	1258
1	1529	855
1	1812	1018
1	2112	1119
1	2372	1275
1	3439	1815
1	4318	2174
1	5159	2628
1	4377	2182

TABLE B5.2

Test:	Stain Series 9339TH, Set B
Storage Time:	2 days
Test Fluid:	Thuricide 32LU
Witness Card Material:	Bac-T-Kote Membrane #1121
*Generation Method:	WDT

No. stains used in sample	Ds (um)	Ddf (um)
1	1856	1272
1	1169	943
1	811	918
1	1095	816
1	1650	1255
1	1896	1652
1	2181	1570
1	3507	2495
1	2880	2154
1	3213	2029

TABLE B6.1

Test:	Stain Series 9339F, Set A
Storage Time:	2 days
Test Fluid:	Foray 48B
Witness Card Material:	Kromekote Cov. White C2S
*Generation Method:	WDT

No. stains used in sample	Ds (um)	Ddf (um)
1	1913	1251
1	2259	1407
1	2401	1524
1	2717	1695
1	3676	2250
1	4128	2523
1	5021	2929
1	4508	2754

TABLE B6.2

Test:	Stain Series 9339F, Set B
Storage Time:	2 days
Test Fluid:	Foray 48B
Witness Card Material:	Bac-T-Kote Membrane #1121
*Generation Method:	WDT

No. stains used in sample	Ds (um)	Ddf (um)
1	1180	1078
1	1735	1204
1	2096	1215
1	2687	1234
1	2188	1882
1	3807	1740
1	2953	2284
1	3362	1585

TABLE B7.1

Test: Solar Degradation Trial 9333TH, Set A
Storage Time: 7 days
Test Fluid: Thuricide 32LU
Witness Card Material: Kromekote Cov. White C2S
*Generation Method: WDT

Hours of Solar Exposure	Drop Mass (mg) from Mass Balance	Drop Mass (mg) from Fluoresence
4	0.70	0.68
4	0.55	0.55
4	0.69	0.70
4	0.49	0.50
4	0.89	0.91
4	0.49	0.52
4	0.60	0.63
0	0.89	0.92
0	0.64	0.66
0	0.59	0.66
0	0.52	0.54
0	0.51	0.51
0	0.47	0.47
0	0.61	0.62

TABLE B7.2

Test: Solar Degradation Trial 9333TH, Set B
Storage Time: 14 days
Test Fluid: Thuricide 32LU
Witness Card Material: Kromekote Cov. White C2S
*Generation Method: WDT

Hours of Solar Exposure	Drop Mass (mg) from Mass Balance	Drop Mass (mg) from Fluoresence
4	0.64	0.52
4	1.09	1.01
4	0.52	0.46
4	0.56	0.53
4	0.72	0.70
4	0.53	0.49
4	0.71	0.71
0	0.55	0.52
0	0.61	0.60
0	0.55	0.55
0	0.49	0.48
0	0.46	0.42
0	0.80	0.78

0	0.64	0.43

TABLE B7.3

Test: Solar Degradation Trial 9333TH, Set C
Storage Time: 28 days
Test Fluid: Thuricide 32LU
Witness Card Material: Kromekote Cov. White C2S
*Generation Method: WDT

Hours of Solar Exposure	Drop Mass (mg) from Mass Balance	Drop Mass (mg) from Fluoresence
4	0.53	0.48
4	0.59	0.48
4	0.55	0.53
4	0.63	0.57
4	0.48	0.43
4	0.72	0.74
4	0.43	0.39
0	0.49	0.38
0	0.71	0.67
0	0.54	0.50
0	0.58	0.53
0	0.64	0.61
0	0.45	0.43
0	0.60	0.52

TABLE B8

Test: Stain Series 9345TH
Storage Time: 7 days
Test Fluid: Thuricide 32LU
Witness Card Material: Kromekote Cov. White C2S
*Generation Method: CAS

No. stains used in sample	Ds (um)	Ddf (um)
14	199	138
18	185	148
6	226	166
12	250	175
10	270	274
7	299	181
10	321	207
8	349	230
5	382	313
5	424	278
6	454	278
5	480	278
4	512	295
6	560	303

6	599	204

TABLE B9

Test:	Stain Series 9345F
Storage Time:	7 days
Test Fluid:	Foray 48B
Witness Card Material:	Kromekote Cov. White C2S
*Generation Method:	CAS

No. stains used in sample	Ds (um)	Ddf (um)
14	206	170
14	224	188
15	248	203
15	277	207
14	301	225
13	327	232
14	353	272
10	375	276
11	401	290
8	430	305
7	466	318
6	488	323
6	520	339
5	549	358
6	611	387

TABLE B10

Test: Stain Series 0002F
Storage Time: 3 days
Test Fluid: Foray 48B
Witness Card Material: Kromekote Cov. White C2S
*Generation Method: PS

No. stains used in sample	Ds (um)	Ddf (um)
8	198	216
10	133	234
11	159	214
9	225	252
11	280	241
9	247	306
10	318	268
10	353	286
6	406	334
7	506	383
6	625	436
4	745	493
3	907	587
2	1068	676
1	1338	775
1	1937	1021
1	2354	1161
1	3346	1412
1	4925	1944
1	2861	1311
1	3813	1644
1	5417	2091
1	1792	948
1	1214	745

TABLE B11

Test:	Stain Series 0036THW
Storage Time:	1 day
Test Fluid:	1:1 Thuricide 32LU/Water
Witness Card Material:	Kromekote Cov. White C2S
*Generation Method:	PS

No. stains used in sample	Ds (um)	Ddf (um)
1	1003	430
1	1132	473
1	1242	353
1	1337	548
1	1442	586
1	1618	728
1	1899	731
1	2269	803
1	2552	879
1	3365	1080
1	3554	1129
1	3886	1241
1	4258	1303
1	4668	1392
1	4900	1433
1	1770	705
1	2634	918
1	1972	722
1	1065	432
1	1184	503
9	257	156
16	315	179
14	351	216
10	394	209
13	189	130
10	455	232
16	127	123
9	507	251
7	624	288

---

* Generation Methods:
1. WDT - wire droplet technique
2. PS - pump sprayer, finger operated (common household item)
3. CAS - compressed air sprayer

APPENDIX C

Density Measurements of
Thuricide 32LV and Thuricide 32LV/Water 1:1 (mass basis) Mixture

C-1. *INTRODUCTION*

In order to convert droplet mass values obtained into their corresponding diameter values, the density of the liquid has to be known. For Foray 48B, its density was provided in the manufacturer's literature.[7] However, this information was not provided by the manufacturer for Thuricide 32LV. Thus, density measurements were made in the present study for both Thuricide 32 LV and an 1:1 (mass basis) mixture of Thuricide 32LV/Water.

C-2. *EXPERIMENTAL PROCEDURE*

Three, 10 ml samples of each solution were each placed into an 10 ml volumetric flask (care being taken to fill the flasks to the mark). A separate dry and clean flask was used for each sample. A Mettler Model AE163 Analytical Balance was used to weight the mass of each solution by difference. The ambient temperature at the time of the measurements was 20^{o}C.

C-3. *RESULTS*

In Table D1 are the mass values for the three samples for each solution, as well as an average value and standard deviation. The accuracy of the solution volume is $\pm$ 1% (as stated on the flasks used).

The estimated accuracy of the density values is four significant digits. However due to variation in temperatures during spraying, the use of only three significant digits would be prudent, 1.15 g/ml for Thuricide and 1.07 g/ml for Thuricide/Water. It was these values that were used (see Section 4) in all calculations in the present work.

TABLE C1

Density Measurements--Neat Solutions 9285TH and 0032THW
Mass Values for 10 ml Solution

	Solution Mass (grams)	
Sample	Thuricide (NS9285TH)	Thuricide/Water (NS0032THW)
1	11.4682	10.7308
2	11.4441	10.6365
3	11.4785	10.6812
Average	11.46 ± 0.02	10.68 ± 0.05
Density (g/ml)	1.146	1.068

APPENDIX D

Sample Calculation to Determine Droplet Freefall Evaporational Losses

D-1. *INTRODUCTION*

As explained in Section 2.2.1, it is possible to determine droplet free fall evaporation using the spectroscopic spreadfactor. The following sample calculation is presented showing how Eq. (2) in conjuction with spreadfactor information is used towards this end:

$$(2) \qquad R_c = 100 \left(\frac{D_{df}}{D_{di}} \right)^3$$

D-2. *SAMPLE CALCULATION*

Assume that a spraying operation using Kromekote witness cards is being conducted with Thuricide 32LV (no dilution) with Rhodamine WT Liquid added as a tracer. The spreadfactor under non-evaporative conditions had already been determined (see Figure 1 and Table 2) for this solution/witness card combination:

$$(D1) \qquad \log_{10} D_{df} = (0.763)\log_{10} D_s + (0.423)$$

A stain having a D_s value of 300 μm was subjected to solvent extraction, and using the laboratory procedure outlined in Section 3.3, D_{di} was found to be 250 μm. It should be remembered that all droplet diameter values determined from the fluorescence measurement of the recovered tracer dye are D_{di} values, not D_{df} (unless non-evaporative conditions are involved, then $D_{di} = D_{df}$). The amount of dye present in the droplet is fixed at the moment of creation and is not lost during droplet evaporation.

Using Eq. (D1), D_{df} is found to be 206 μm for $D_s = 300$ μm. Putting D_{di} and D_{df} into Eq. (2):

(2) $R_c = 100 \left(\frac{206}{250} \right)^3 = 55.9\%$ or 44.1% evaporation loss

If there had been no evaporational losses of the 250 μm droplet ($D_{di} = D_{df}$) then D_s should of been 388 μm instead of 300 μm (using Eq. (D1)). Had droplet evaporation not been accounted for, the error in D_{df} would have been 17.6% (206/250 μm).

Figure 1

Spreadfactor Regression, Thuricide 32LV

Stains made on Kromekote Paper

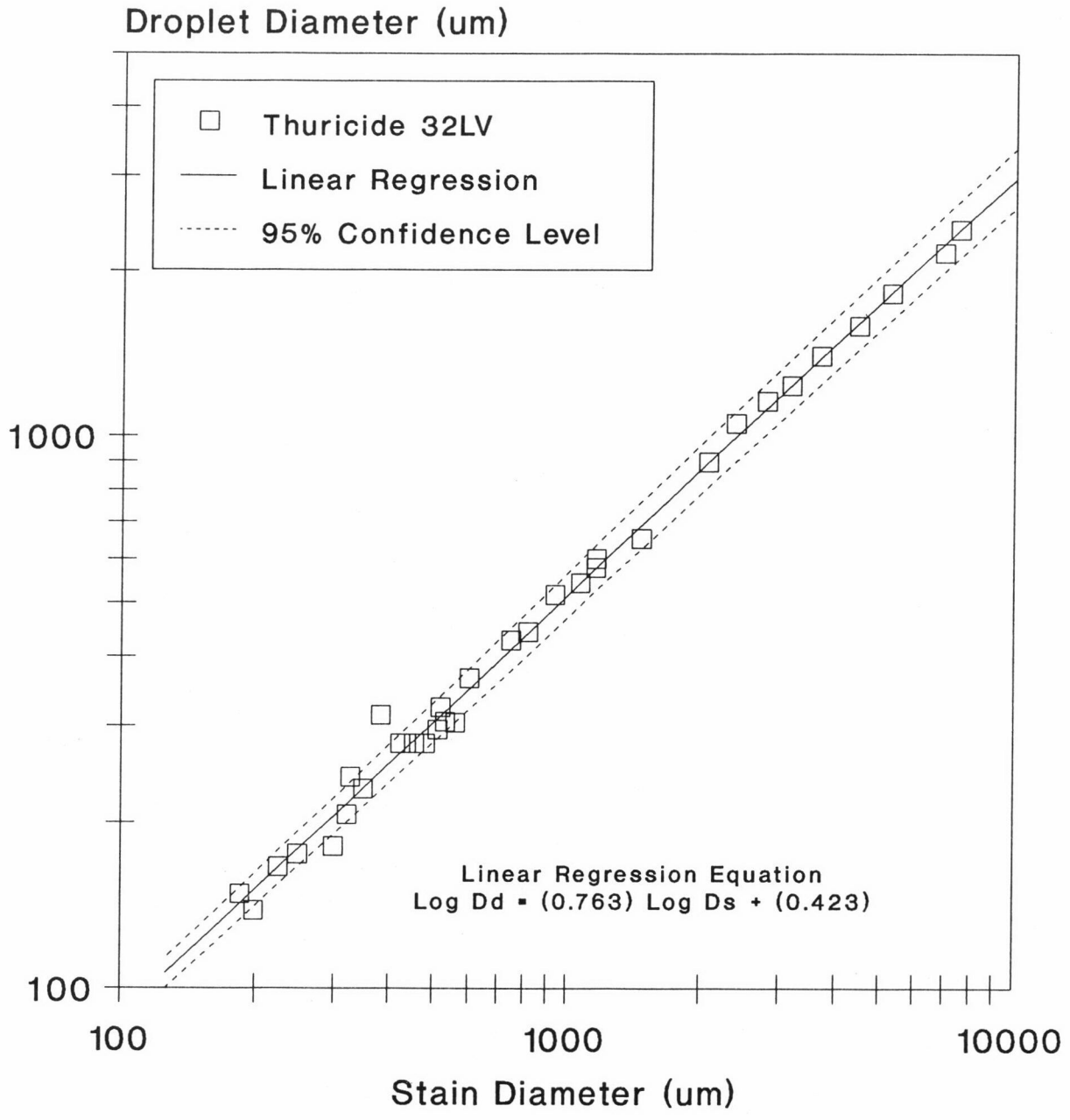


Figure 2

Spreadfactor Regression, Thuricide/Water

Stains made on Kromekote Paper

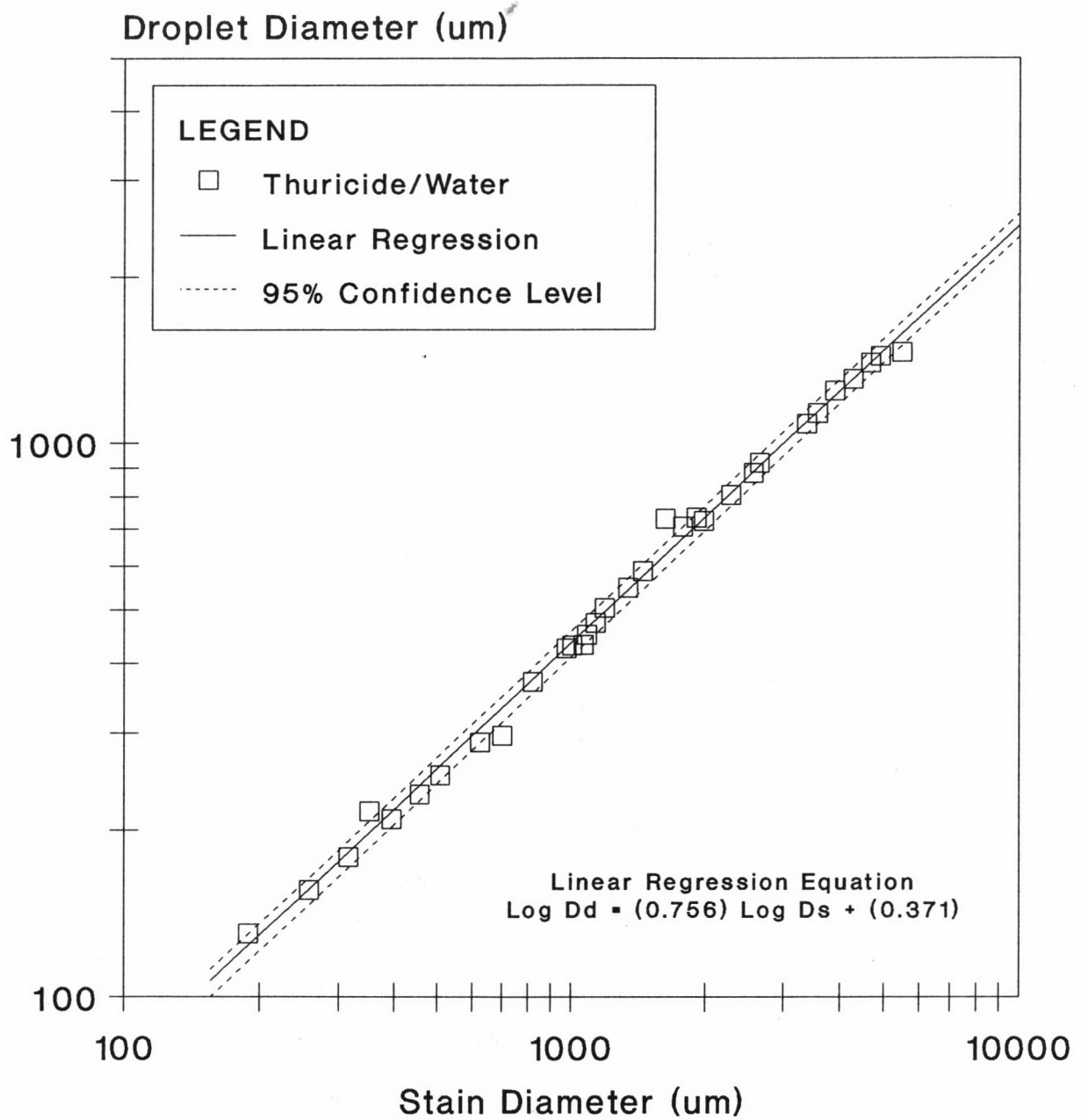


Figure 3

Spreadfactor Regression, Foray 48B

Stains made on Kromekote Paper

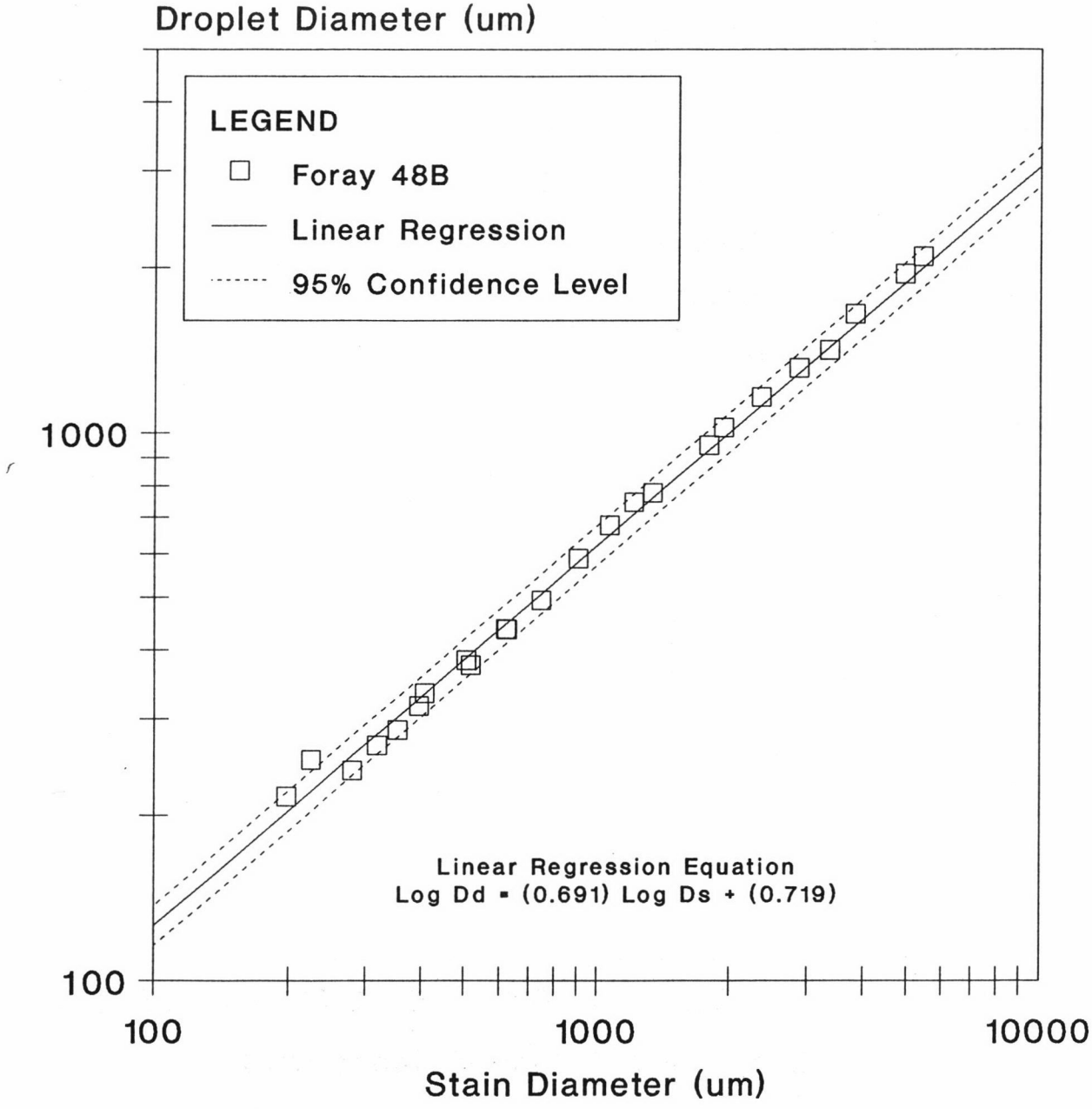
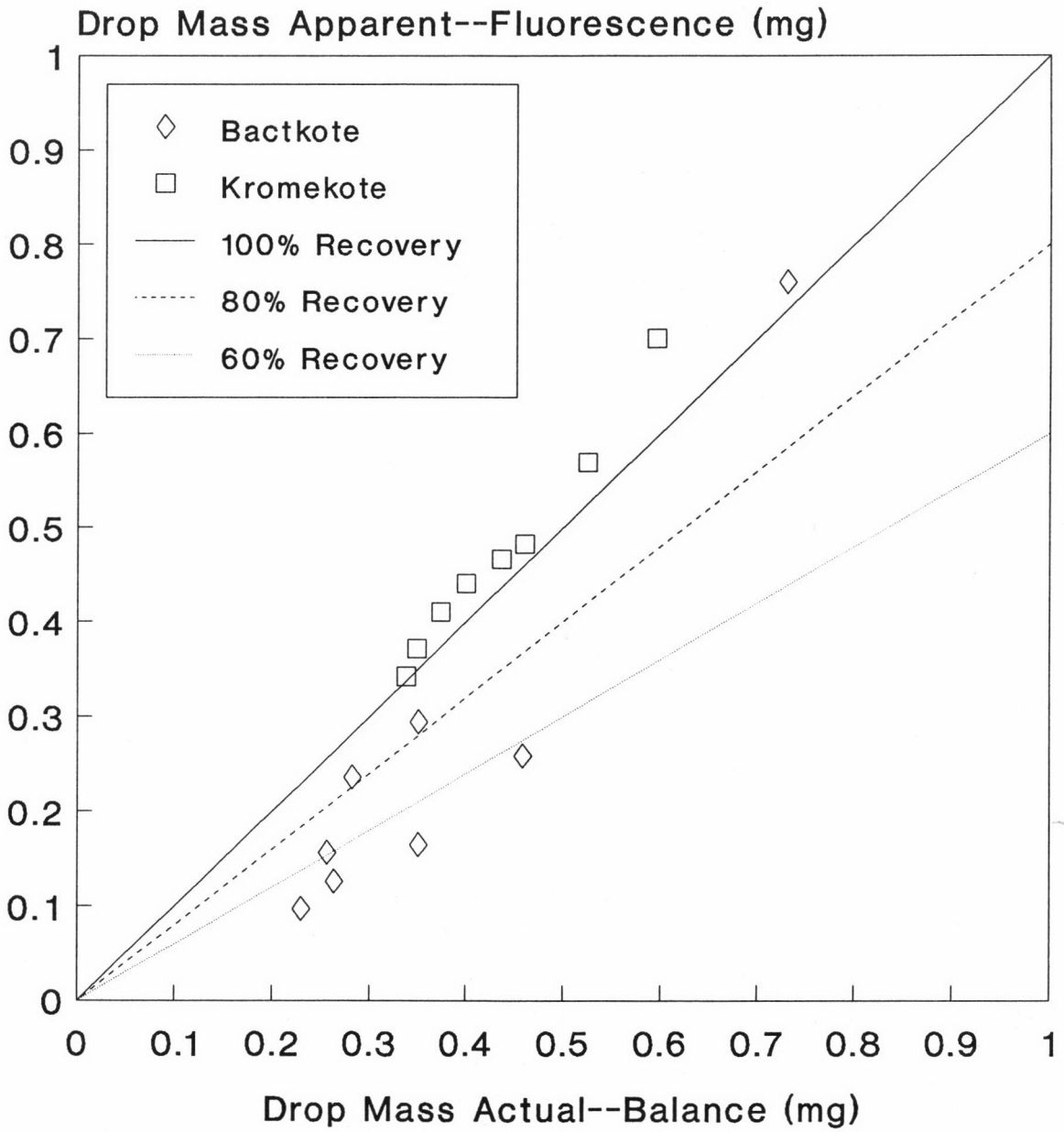


Figure 4

Dye Recovery from Witness Cards

Comparison of Apparent/Actual Drop Mass



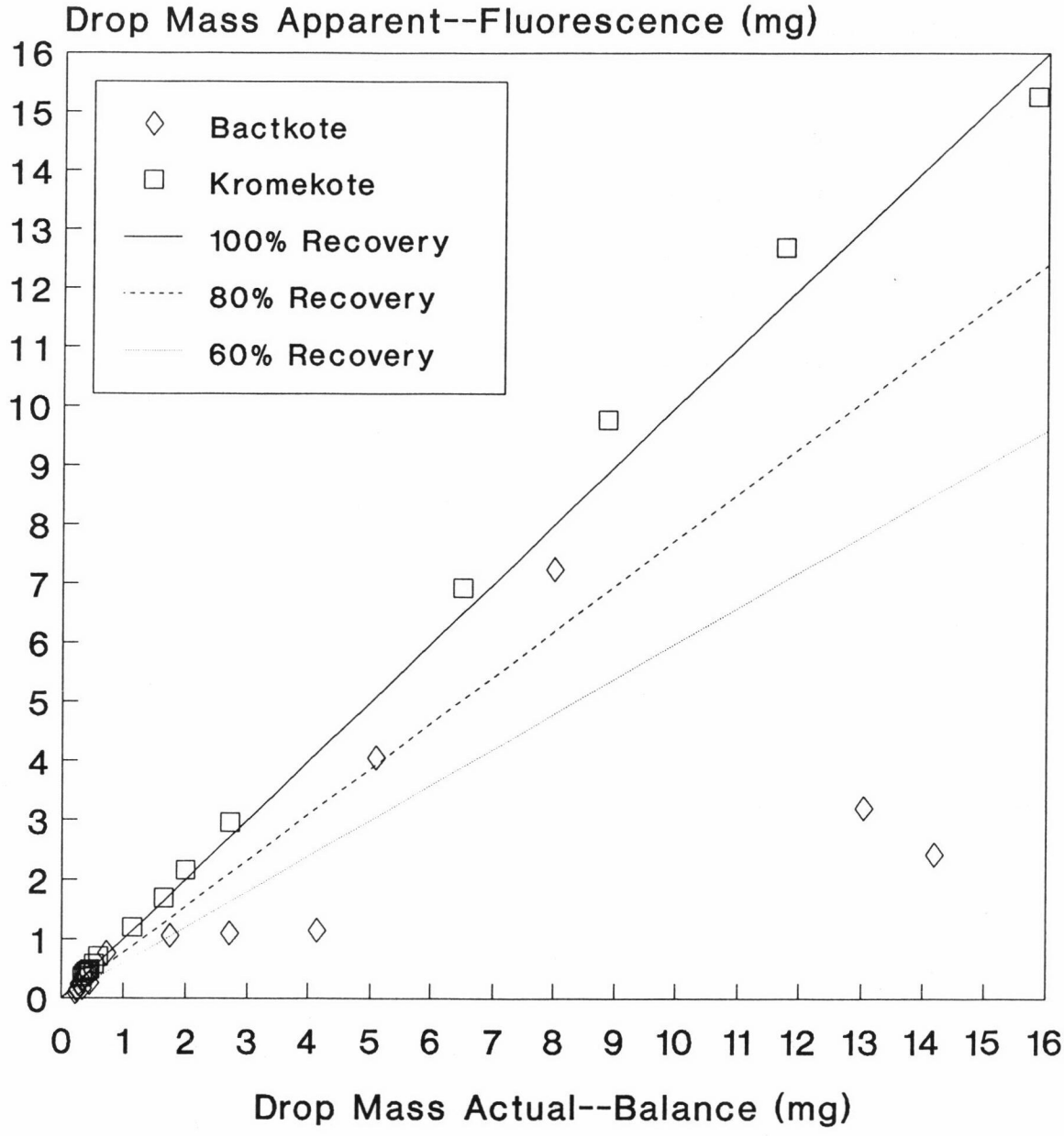
LOW REGION

Rhodamine WT Liquid/Foray 48B Solution

Figure 5

Dye Recovery from Witness Cards

Comparison of Apparent/Actual Drop Mass

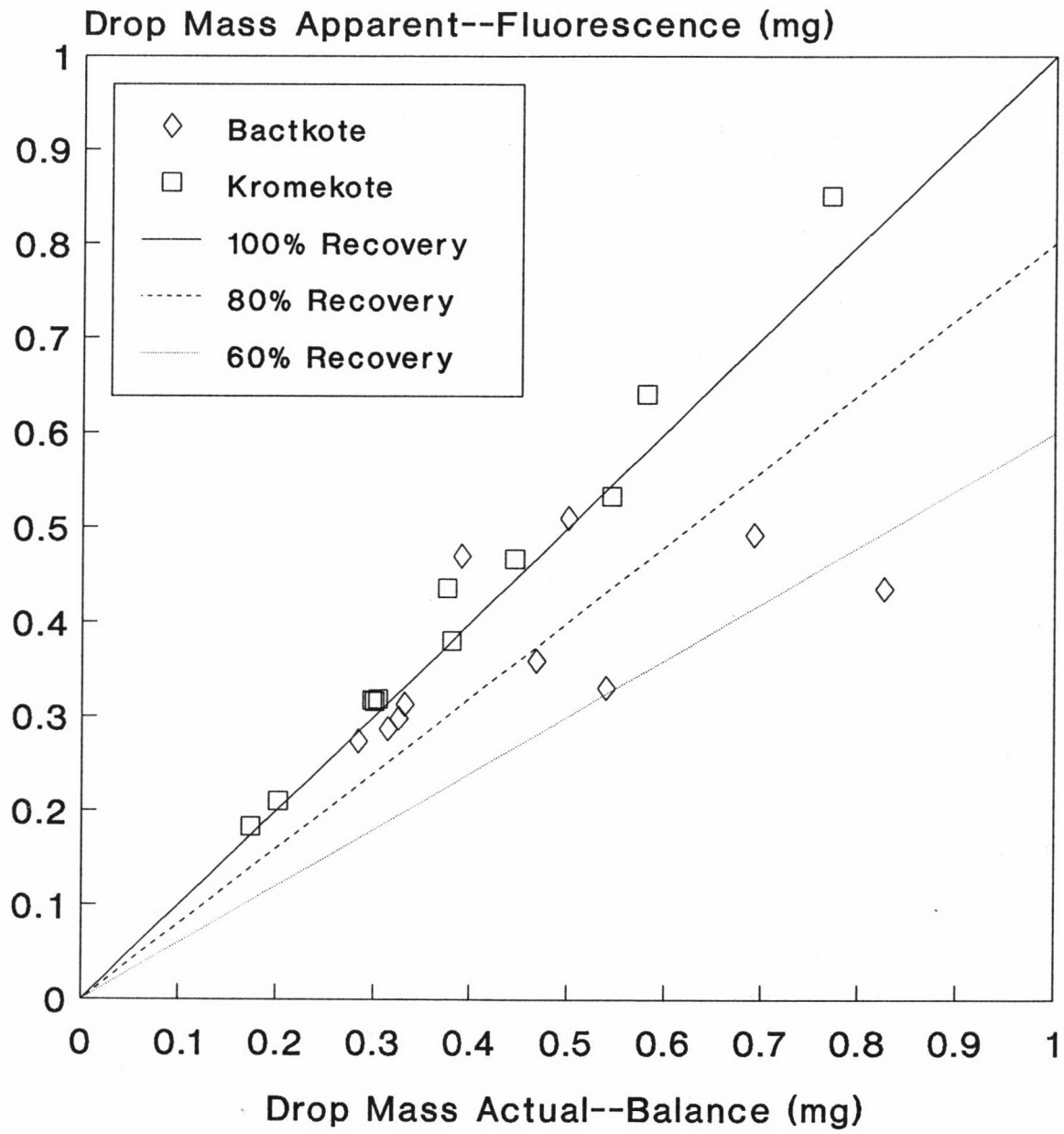


ENTIRE REGION
Rhodamine WT Liquid/Foray 48B Solution

Figure 6

Dye Recovery from Witness Cards

Comparison of Apparent/Actual Drop Mass

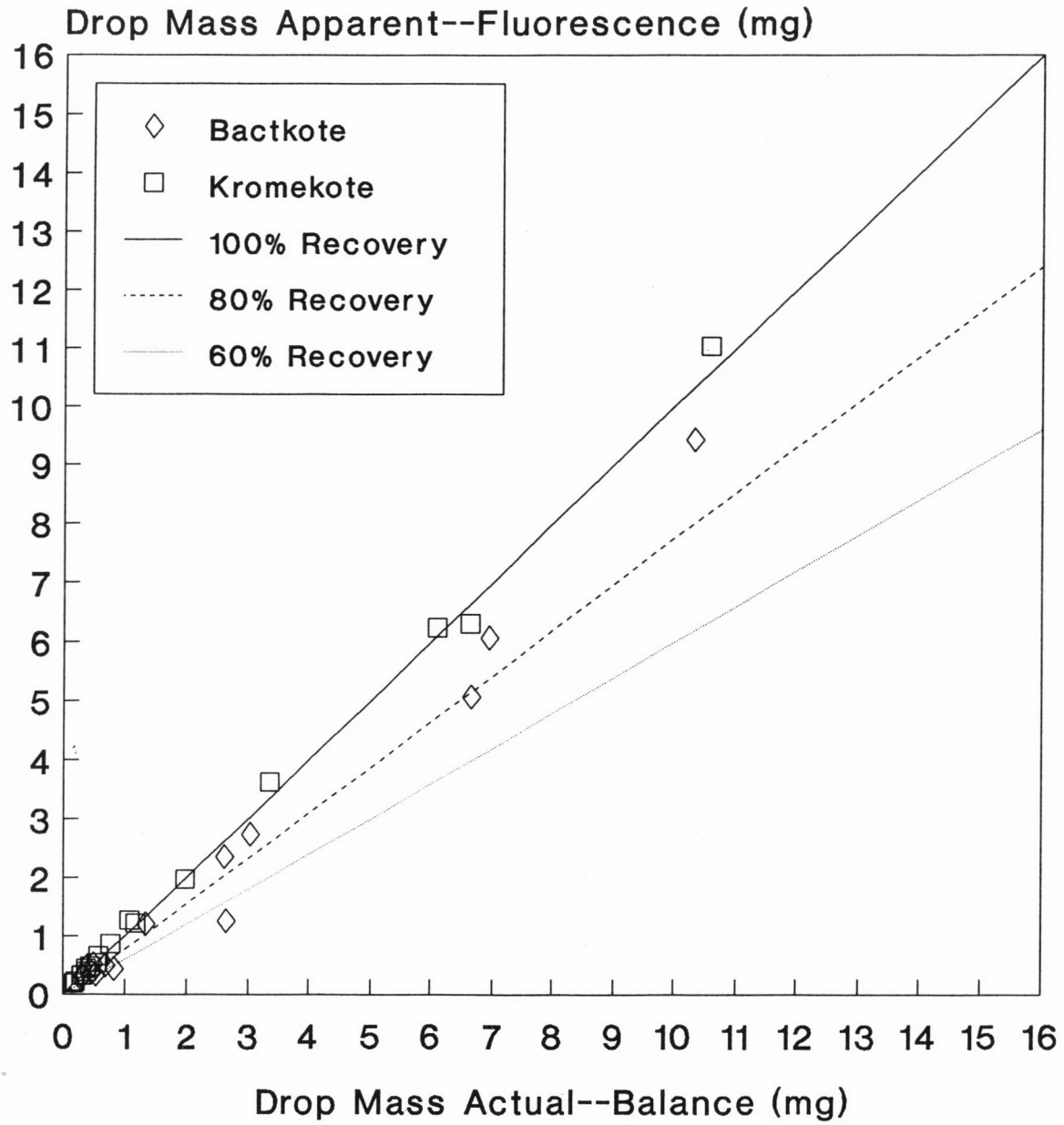


LOW REGION
Rhodamine WT Liquid/Thuricide 32LV

Figure 7

Dye Recovery from Witness Cards

Comparison of Apparent/Actual Drop Mass

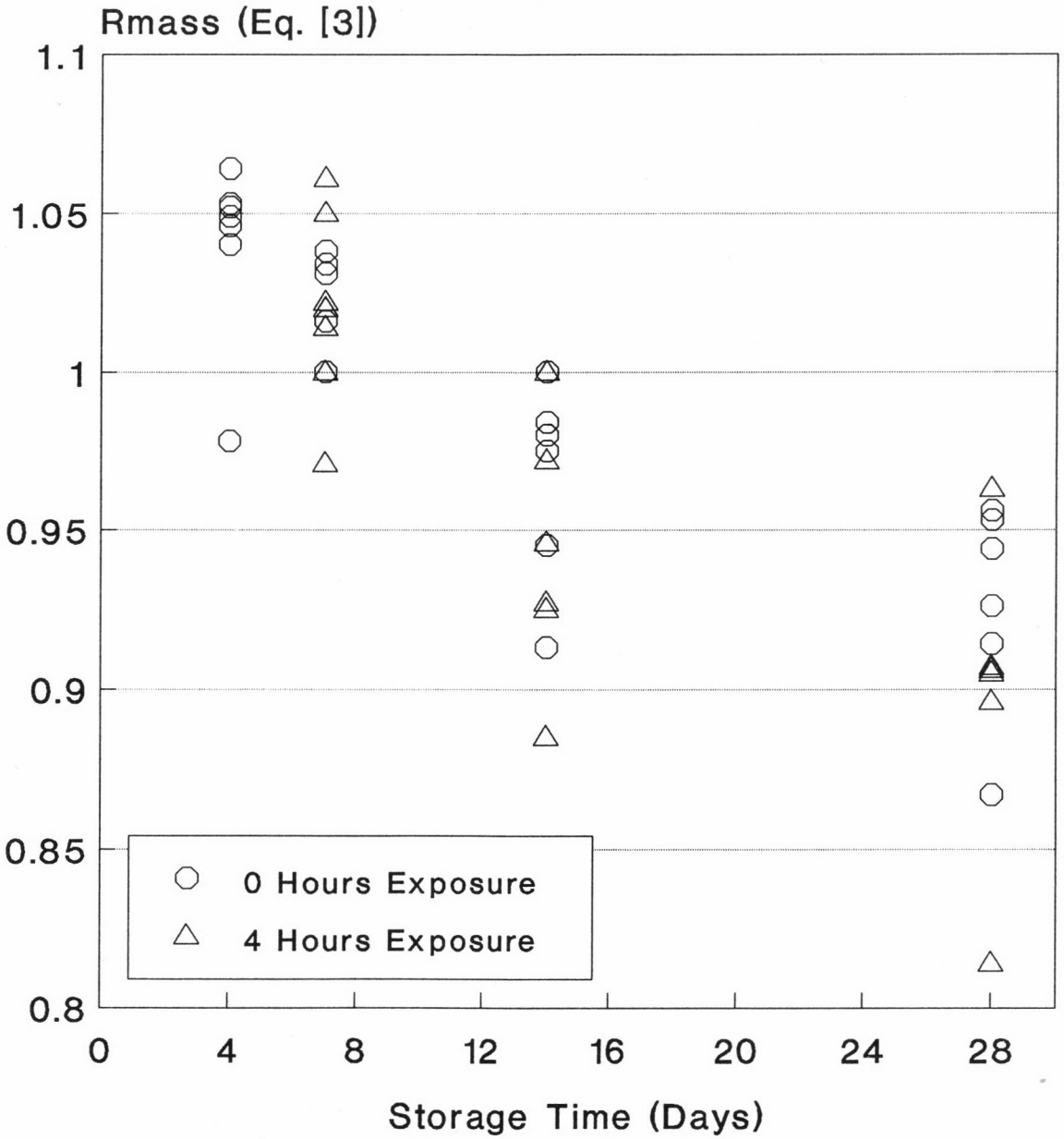


ENTIRE REGION
Rhodamine WT Liquid/Thuricide 32LV

Figure 8

Dye Degradation--Rhodamine WT Liquid

Thuricide 32LV on Kromekote Paper

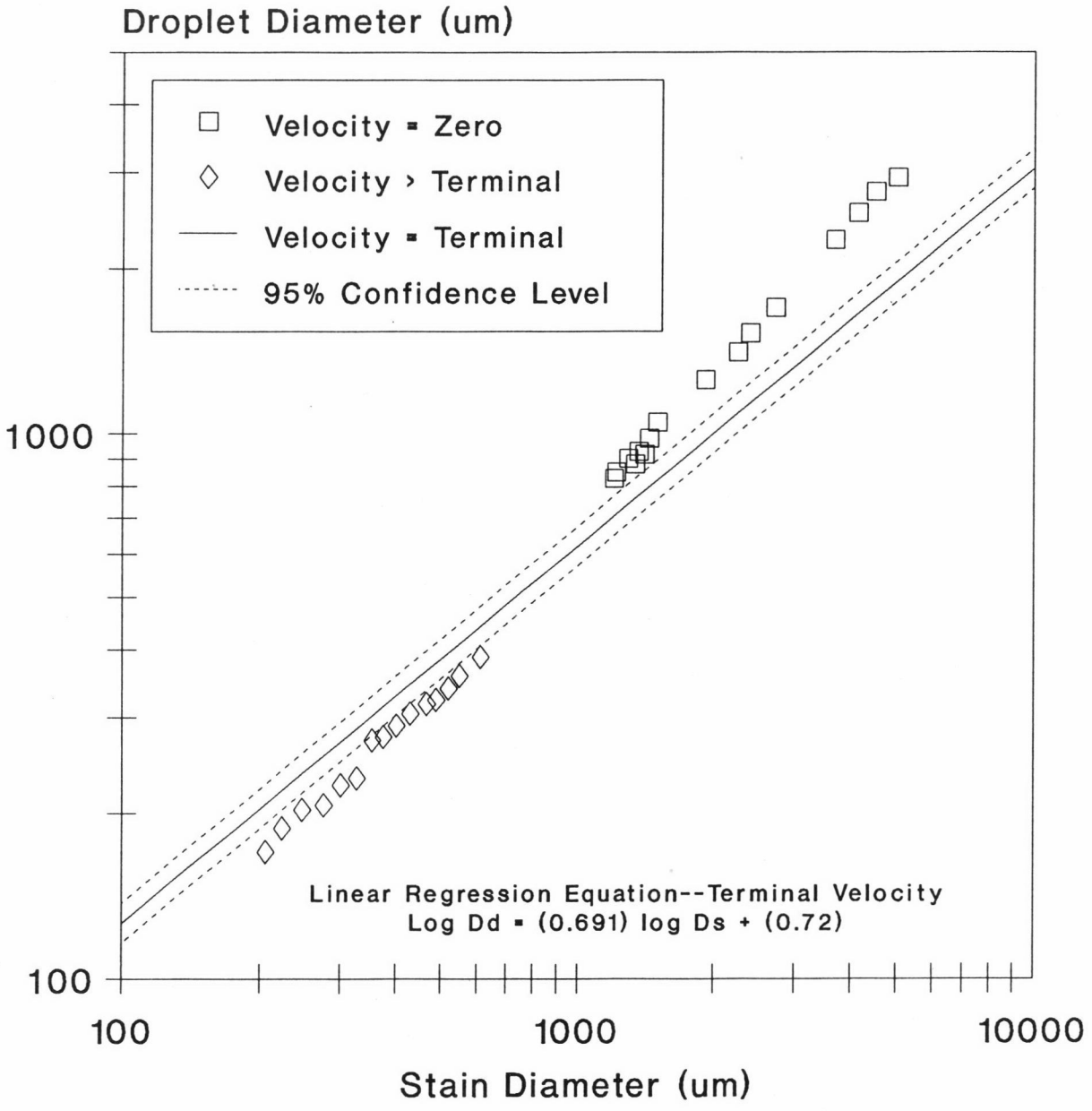


0 and 4 Hours of Solar Exposure
Rmass--Ratio Apparent/Actual Drop Mass

Figure 9

Effects of Droplet Impact Velocity

Foray 48B on Kromekote Paper



Curve and Equation for Terminal Velocity
Droplets Taken from Figure 3--Foray 48B

APPENDIX E

Scanning Electron Microscope Photographs of Test Materials

E-1. *INTRODUCTION*

Scanning electron microscopy was used to view the surface characteristics of some of the test materials in the present study. The microscopy work was conducted with a J.E.O.L. model JSM-35CF at magnifications ranging from x200 to x3600. Photomicrographs were produced with Polaroid type #55 Black and White film. At the bottom of each photomicrograph (shown in Figures E1-E7) are the instrument parameters, date, and a micron marker.

E-2. *PHOTOMICROGRAPHS*

E-2.1 *Figure E1: Foray 48B Stain on Bactkote Paper*

This stain was generated in SS9339F using Foray 48B and tracer dye solution. BTK spores can be seen protruding outward from the surface though still covered with a layer of inert material. A dye halo, 50 μm in thickness, can be seen surrounding the main section of the stain. The dye halo is not readily observable with the naked eye.

E-2.2 *Figure E2: Foray 48B Stain on Kromekote Paper*

This stain was generated in SS9345F using Foray 48B and tracer dye solution. Not as many spores are visible protruding beneath the surface, and the dye halo is only 20 μm in width.

E-2.3 *Figure E3: Membrane Surface of Bactkote Paper*

Bactkote paper has a porous and sponge-like surface on its membrane side.

E-2.4 *Figure E4: Surface of Kromekote Paper*

Kromekote paper is densely packed with very small imperfection (less than 1 μm in size). Some dye (Rhodamine WT Liquid) splotches can also be seen in the left center of the figure.

E-2.5 *Figure E5: Bacillus thuringiensis kurstaki Spore*

A close-up view of an individual BTK spore is presented.

E-2.6 *Figure E6: Edge of Foray 48B Stain on Kromekote Paper*

The boundary between fill (left to center), dye halo (center to center right), and the paper background (far right) is presented in this figure. The granular properties of the inert material in the pesticide can be seen.

E-2.7 *Figure E7: Inert (Filler) Material in Foray 48B*

A close-up view of the inert (filler) material is presented in the figure. The granular properties of the inert material in the pesticide are readily apparent.

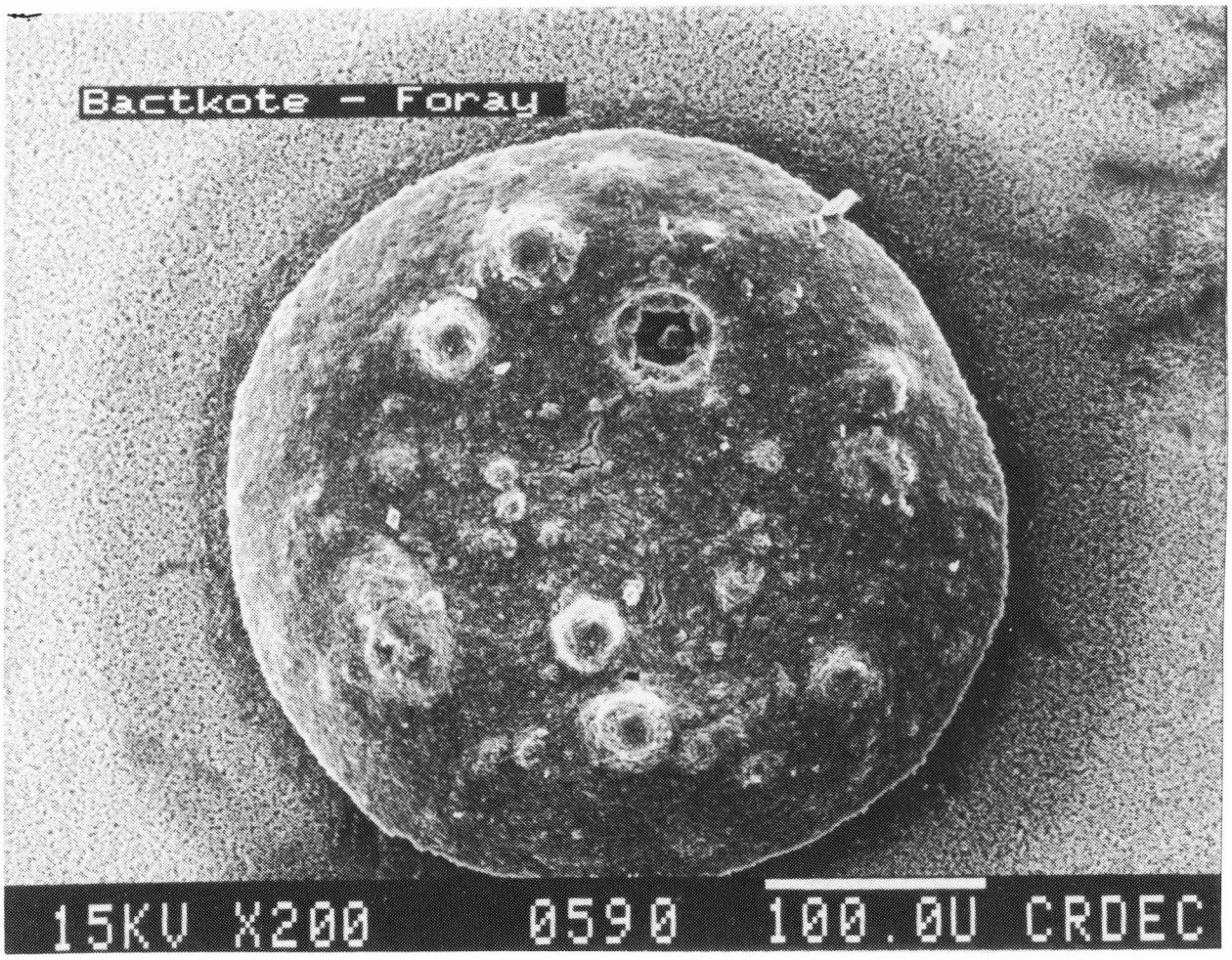


Figure E1 - Foray 48B stain on Bactkote paper.

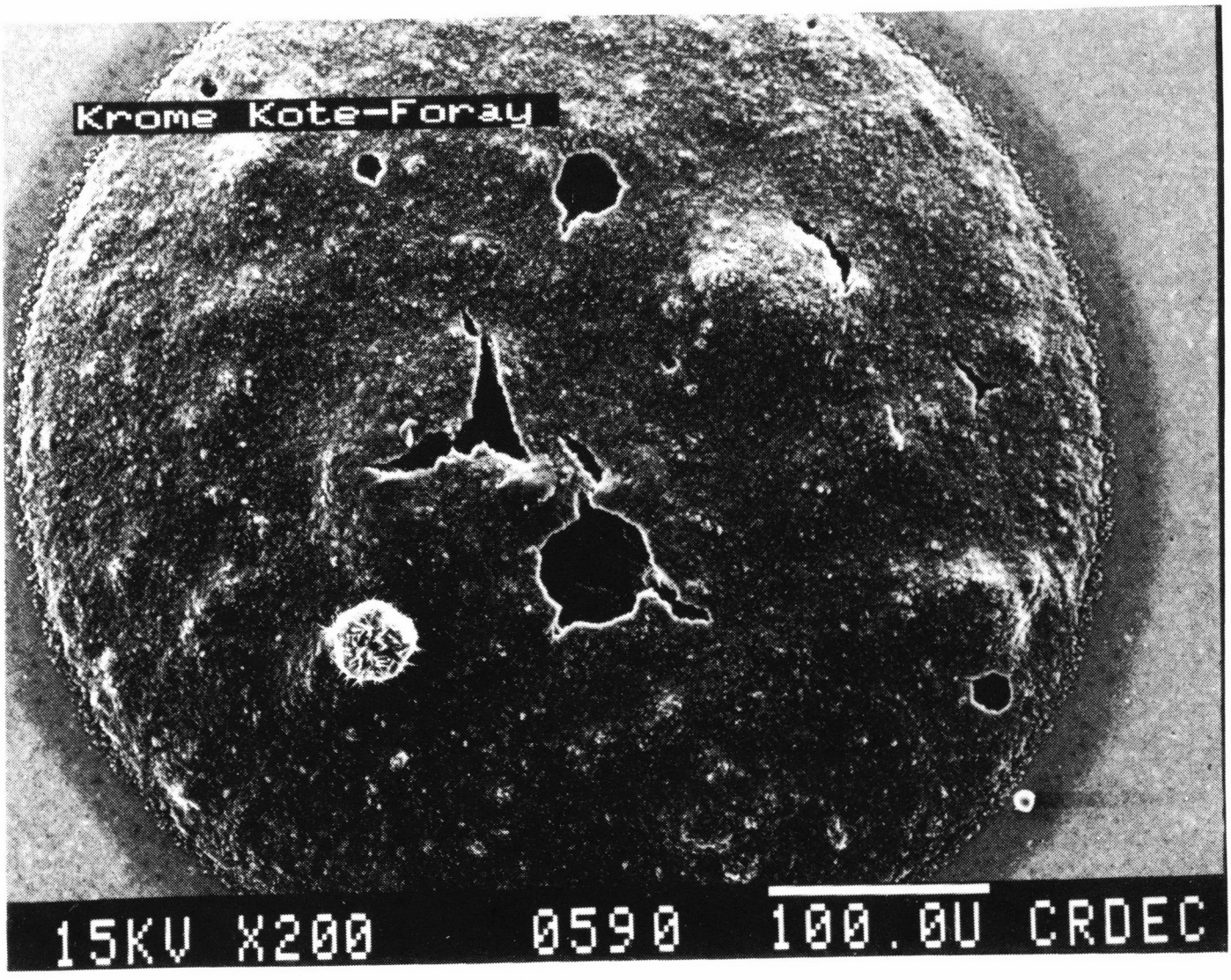


Figure E2 - Foray 48B stain on Kromekote paper.

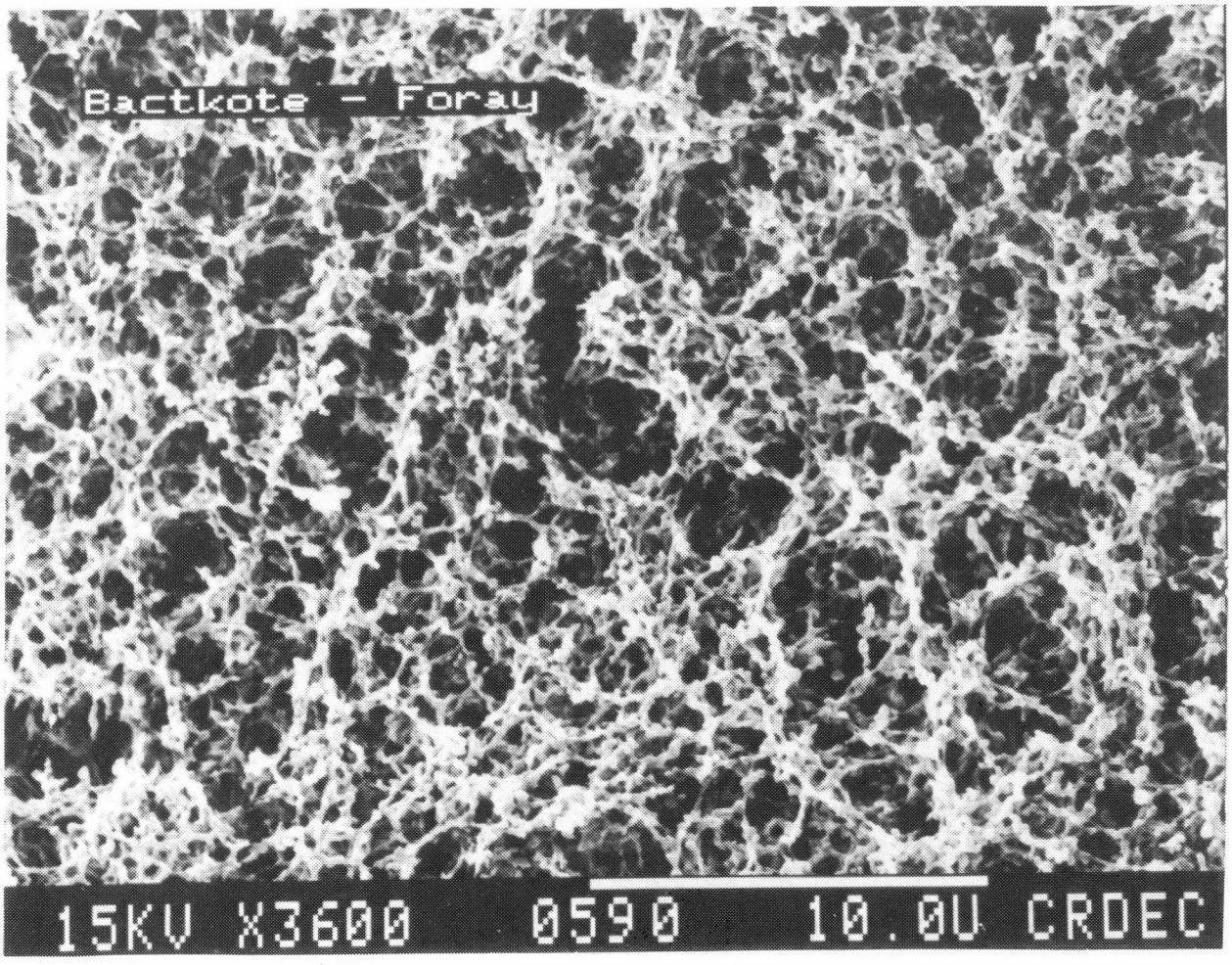


Figure E3 - Membrane surface of Bactkote paper.

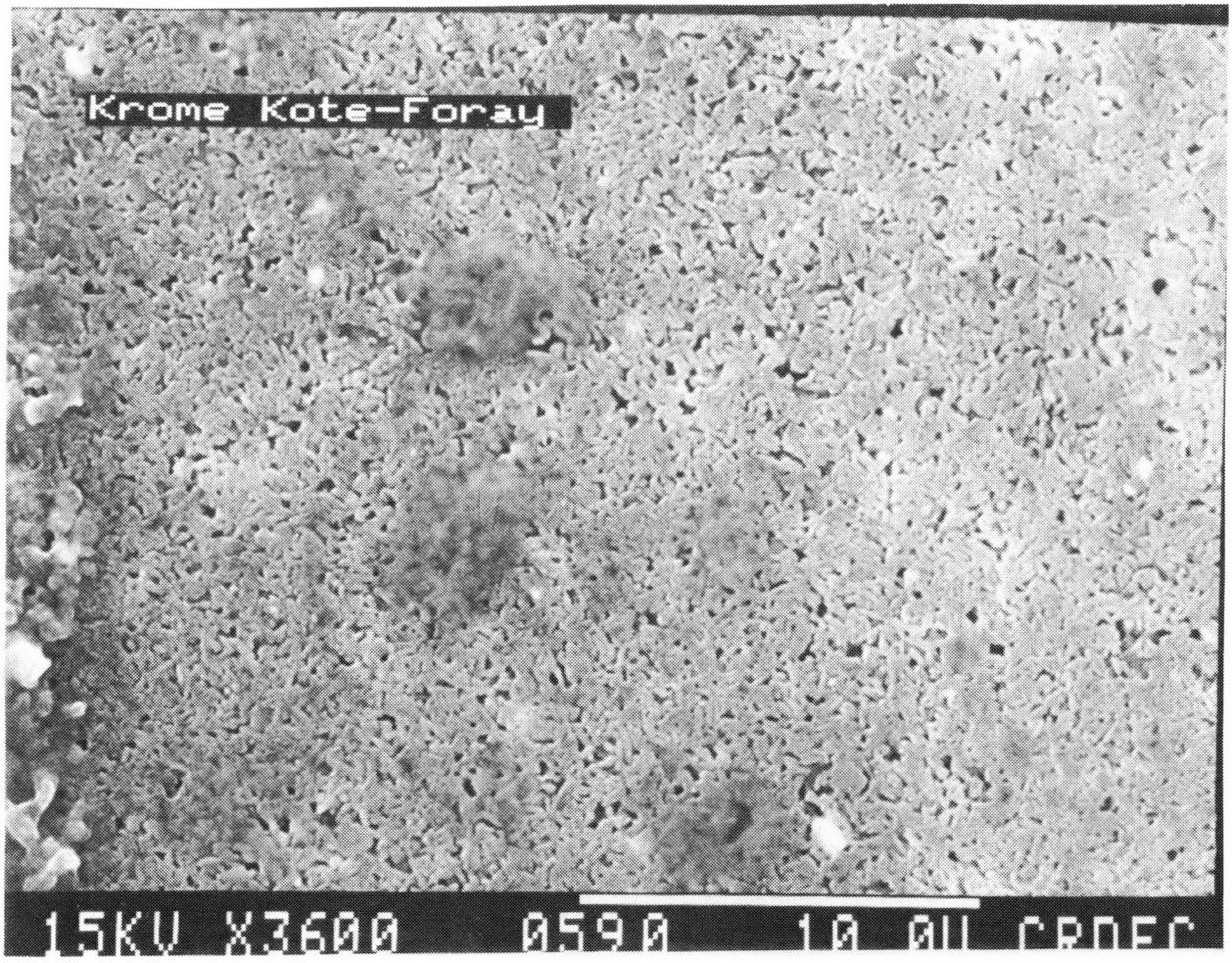


Figure E4 - Surface of Kromekote paper.

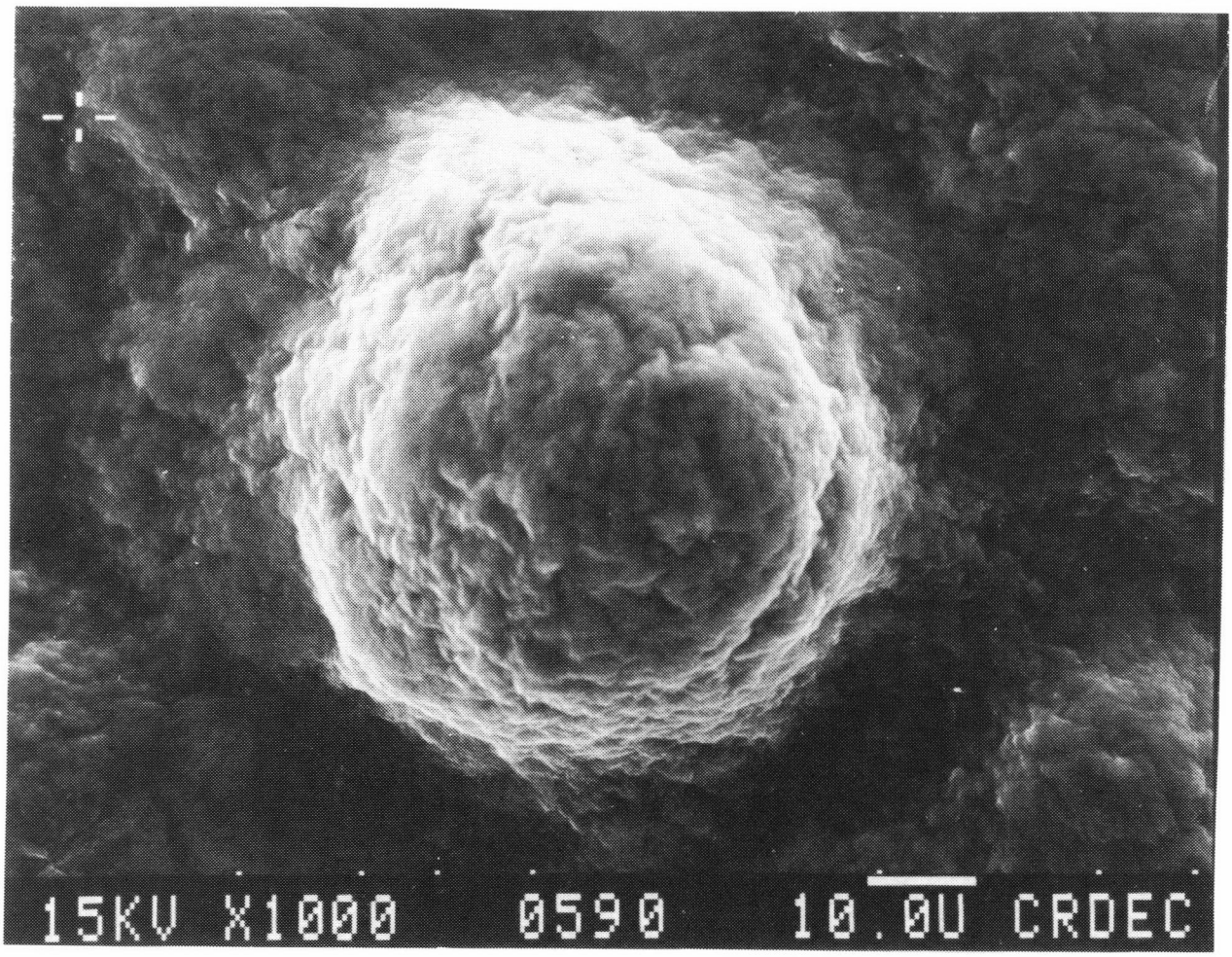


Figure E5 - Bacillus thuringiensis kurstaki spore.

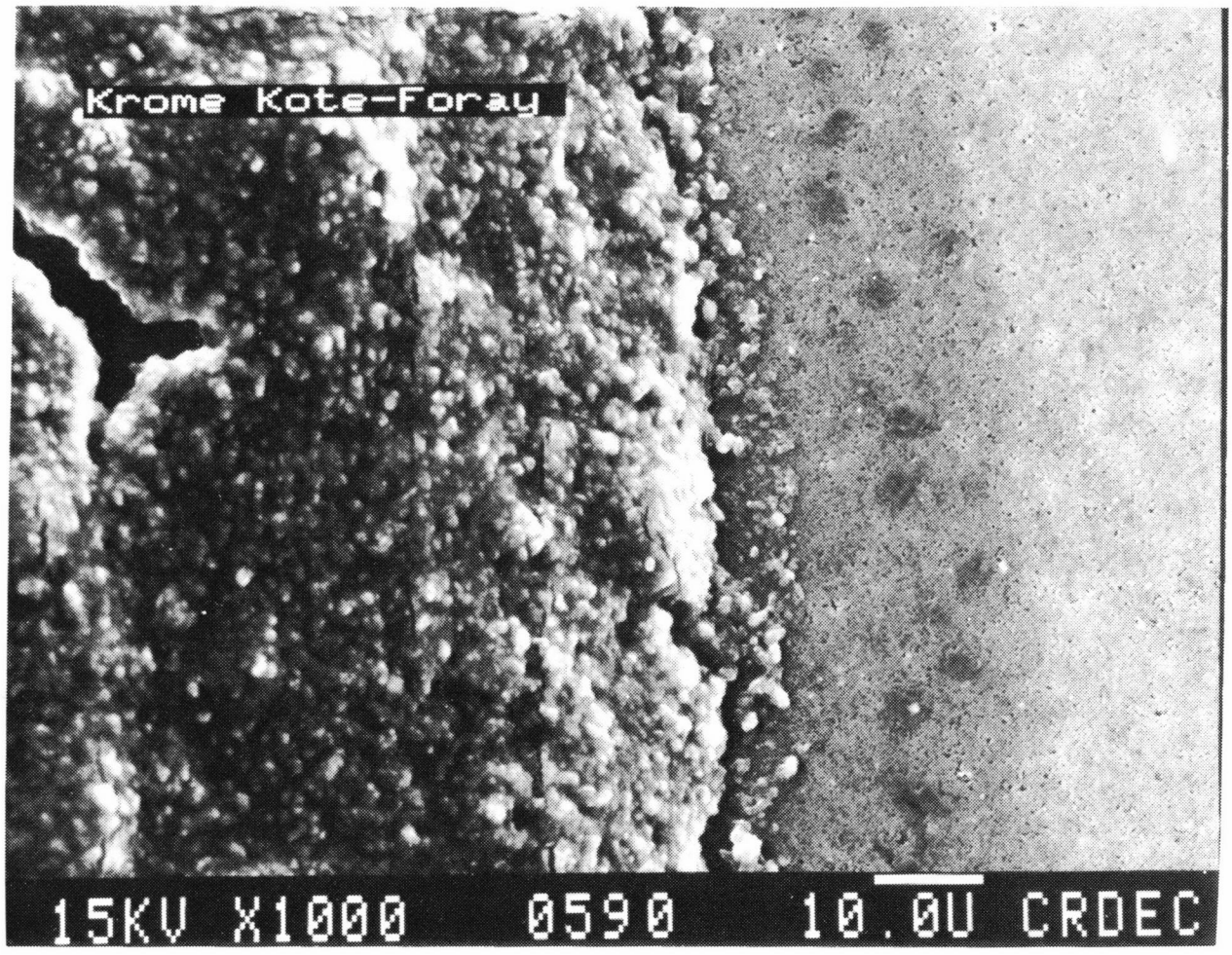


Figure E6 - Edge of Foray 48B stain on Kromekote paper.

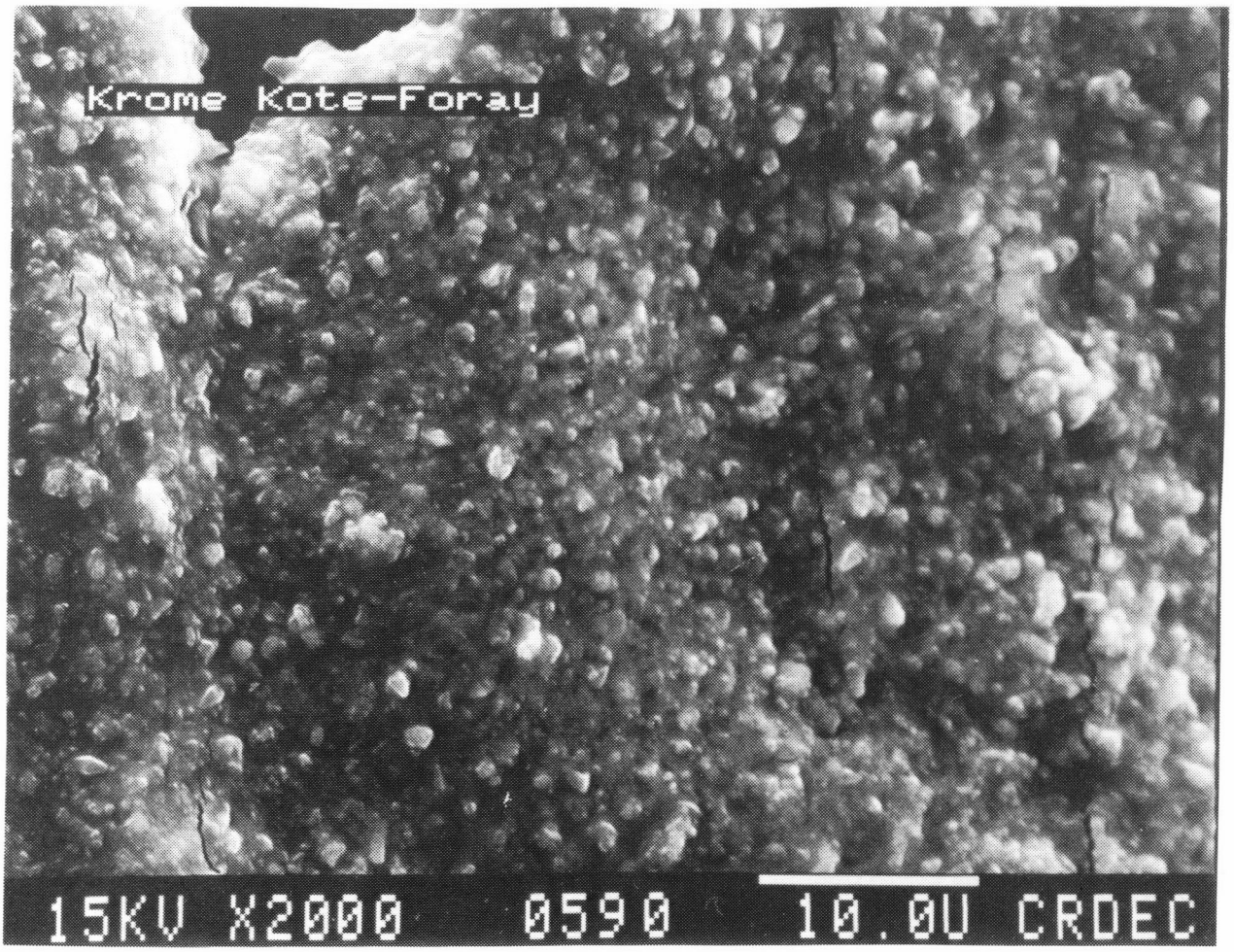


Figure E7 - Inert (filler) material in Foray 48B.

Notes:

INTERMOUNTAIN RESEARCH
STATION
324-25th Street
Ogden, UT 84401